# *BACTERIOLOGICAL PROFILE OF DIABETIC FOOT ULCERS WITH SPECIAL REFERENCE TO $HbA_1C$ LEVELS.*

**Dissertation submitted in**

**Partial fulfillment of the regulations required for the award of**

**M.D. DEGREE**

**In**

**MICROBIOLOGY – BRANCH IV**

**The Tamil Nadu**

## Dr. M.G.R. Medical University

**Chennai**

**April -2013**

# DECLARATION

I, **Dr.A.Shahjahan** solemnly declare that this dissertation entitled **"Bacteriological profile of diabetic foot ulcers with special reference to $HbA_1c$ levels"** was done by me at Coimbatore Medical College, Coimbatore during the period from March 2009 to September 2010 under the supervision of **ANBU.N. ARAVAZHI, M.D.,** former Professor and Head, Department of Microbiology and under the guidance of **DR.K.RAJENDRAN, B.Sc., M.D.,** Professor and Head, Department of Microbiology Coimbatore Medical College, Coimbatore.

This dissertation is submitted to The Tamilnadu Dr.M.G.R. Medical University, Chennai towards the partial fulfillment of the requirement for the award of M.D. Degree (Branch- lV) in Microbiology to be held in April 2013.

I have not submitted this dissertation on any previous occasion to any University for the award of any degree.

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Date**:** **Dr. Shahjahan**

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*Name of the Candidate* : DR. A. SHAHJAHAN

*Course* : M. D. MICROBIOLOGY

*Period of Study* : 2007 - 2010

*College* : COIMBATORE MEDICAL COLLEGE

*Dissertation Topic* : BACTERIOLOGICAL PROFILES OF DIABETIC FOOT ULCERS WITH SPECIAL REFERENCE TO HBA1C LEVELS.

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This is to certify that the dissertation entitled **"Bacteriological profile of Diabetic foot ulcers with special reference to $HbA_1C$ levels"** is a bonafide work done by **Dr.Shahjahan**, Post graduate student in the Department of Microbiology, under the supervision of **DR.ANBU.N. ARAVAZHI, M.D.,** former Professor and Head, Department of Microbiology, Coimbatore Medical College and under the guidance of **DR.K.RAJENDRAN, B.Sc., M.D.,** Professor and Head, Department of Microbiology Coimbatore Medical College, Coimbatore, in fulfillment of the regulations of the Tamilnadu Dr.M.G.R. Medical University towards the award of M.D. Degree (Branch- lV) in Microbiology.

**Professor& HOD,**
Department of Microbiology

**DEAN**
Coimbatore Medical College & Hospital

## ACKNOWLEDGEMENT

I extend my heartfelt gratitude to our Dean Dr.R.Vimala M.D., Coimbatore Medical College and Hospital for permitting me to complete and submit this study.

I extend my gratitude to our former Dean I/C Dr. Kumaran, MS. MCh, Coimbatore Medical College for allowing me to carry out this study.

I express my sincere thanks to my guide, Dr. Rajendiran MD, Professor and Head, Department of Microbiology, Coimbatore Medical College for his timely advice, sustained guidance and constant support throughout my studies.

I express my profound sense of gratitude to Dr. Anbu N Aravazhi. Former Professor and Head, Department of Microbiology, Coimbatore Medical College for offering his impeccable guidance in preparing this dissertation.

I wish to express my sincere thanks to Dr. Govindarajan, M.S., Mch., and the Department of Surgery- Coimbatore Medical College, for their kind co-operation with me in sample collection.

I wish to thank Dr.Shankar, Assistant Professor, Department of Microbiology, Coimbatore Medical College, for guiding me with constant encouragement.

I am indebted to Dr N.Bharathi Santhose for his kind help and advice.

I wish to extend my special thanks to Dr.Deepa, Dr Padmini and Dr.Vasuki of the Department of Microbiology for their constant encouragement and advice.

I sincerely thank my colleagues and the technical staff for their support.

I am extremely thankful to the patients who helped me in my study in spite of their pain and sufferings.

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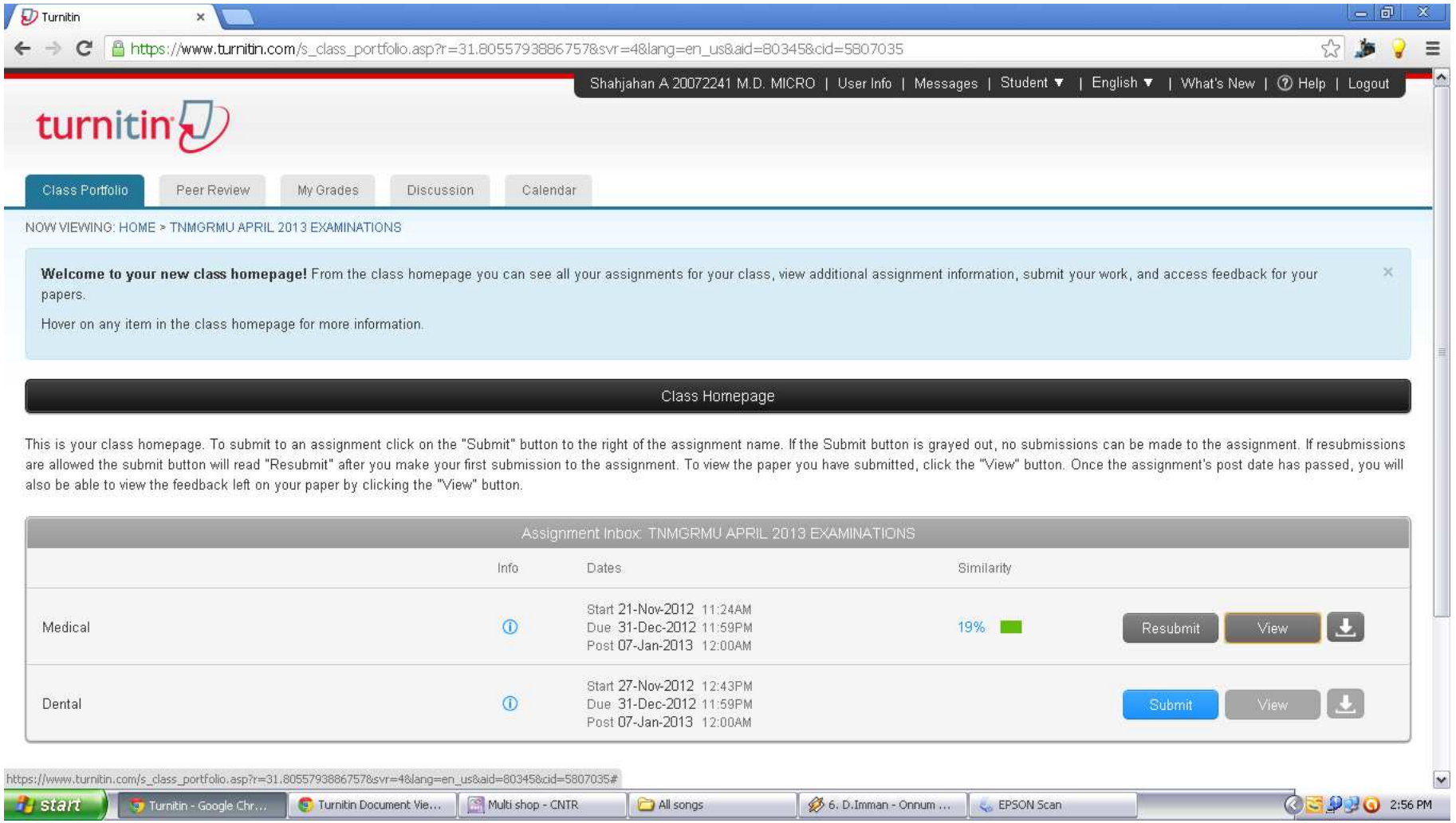
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BACTERIOLOGICAL PROFILE OF DIABETIC FOOT ULCERS WITH SPECIAL REFERENCE TO HbA1C LEVELS. Dissertation submitted in Partial fulfillment of the regulations required for the award of M.D. DEGREE In MICROBIOLOGY – BRANCH IV The Tamil Nadu Dr. M.G.R. Medical University Chennai April -2013 DECLARATION I, Dr.A.Shahjahan solemnly declare that this dissertation entitled “Bacteriological profile of diabetic foot ulcers with special reference to HbA 1 c levels” was done by me at Coimbatore Medical College, Coimbatore during the period from March 2009 to September 2010 under the supervision of ANBU.N. ARAVAZHI, M.D., former Professor and Head, Department of Microbiology and under the guidance of...

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# LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| 1 | ATCC | American Type Culture Collection |
| 2 | E. coli | Escherichia coli |
| 3 | $H_2S$ | Hydrogen sulphide |
| 4 | CLSI | Clinical Laboratory Standards Institute |
| 5 | MR | Methyl Red |
| 6 | VP | Voges Proskauer |
| 7 | HT | Hypertension |
| 8 | DM | Diabetes Mellitus |
| 9 | BSL | Blood Sugar Level |
| 10 | $HbA_1C$ | Glycosylated Hemoglobin $A_1C$ |
| 11 | CONS | Coagulase Negative Staphylococci |
| 12 | MRSA | Methicillin Resistant Staphylococcus aureus |
| 13 | MSSA | Methicillin Sensitive Staphylococcus aureus |
| 14 | PBP | Penicillin Binding Protein |
| 15 | MHA | Mueller Hinton Agar |
| 16 | DFI | Diabetic Foot Infection |

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## INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder affecting a large segment of population and also a major public health problem[1]. Diabetes is rightly called a “disease of complications” and “Iceberg disease”. India homes 33 million diabetics, ranking highest in the world and has a prevalence of about 8% in urban India. Twenty percent of all diabetic complications involve feet[2]. Globally, the prevalence of diabetes is expected to rise from a current estimate of 150 – 220 million in 2010 to 300 million in 2025[3, 4]. The number of people with diabetes is increasing due to population growth, ageing, urbanization, increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes now and the number of people to be affected in future is important to allow rational planning and allocation of resources.

Two major factors are considered important in development of the ‘diabetic foot’[14, 15.]

1. Peripheral neuropathy causing sensory impairment and weakness of intrinsic muscles of the foot and joint that leads to foot deformities.
2. Macro and microangiopathy occurring frequently and leading to ischemia of foot tissues.

Wounds become infected five times more often in diabetics than in non-diabetic patients. Selecting appropriate antimicrobial therapy for diabetic foot infections requires knowledge of likely etiologic agents[5]. The most important characteristic of diabetic foot infection is its polymicrobial nature, and frequent involvement of anaerobes synergistically with aerobes[1, 11].

The common aerobic organisms encountered are S. aureus, Proteus species, Pseudomonas, Escherichia coli, Klebsiella species, Coagulase Negative Staphylococci etc. Pepto-streptococcus species, Bacteroides melaninogenicus and Bacteroides fragilis are commonly isolated anaerobes [6, 16]. The Incidence of aerobic infection is more in lower grades of Wagner's classification. As the grade increases anaerobic infections are encountered frequently[17, 31].

About 10-30% of diabetic patients with foot ulcers will eventually progress to amputation, which may be minor (foot sparing) or major (amputation)[5]. Conversely, an infected foot ulcer precedes ~60% of amputations, making infections perhaps important proximate cause of this tragic outcome[5,]. Mild or non-limb threatening infections can be treated with oral antibiotics, surgical debridement of necrotic tissue, local wound care and close surveillance for progression of infection thus preventing the emergence of complications[10].

In spite of a multidisciplinary foot-care team to optimize foot care, deleterious effects of infection on soft tissue and bone continue to be a major problem in diabetic patients[61]. Progress of infection is usually associated with delayed diagnosis, underestimation of the extent of infection, and inappropriate antimicrobial therapy [11].

The rate of infection parallels blood glucose levels. Blood glucose binds to haemoglobin in red blood cells to form glycosylated haemoglobin. ($HbA_1C$). $HbA_1C$ levels depend on blood glucose concentrations. $HbA_1C$ can be used as a time average index of the blood glucose concentration to which Haemoglobin has to be exposed reflecting the glycemic history in the previous two to three months[54].

Glycemic control is the prime factor in controlling the development of diabetic complications. Poor glycemic control in diabetes has serious complications. Each 2% increase in the level of $HbA_1C$ increases the risk of lower extremity ulcer by 1.6 times and the risk of lower extremity amputation by 1.5 times[63].

The present study was undertaken to assess the role of aerobic bacteria in causation of diabetic foot ulcers. Though anaerobic bacteria are also encountered in diabetic infections, isolation of anaerobes was not feasible due to lack of facilities.

This study has been carried out to detect the antibiotic sensitivity pattern of the isolates and MRSA. The antimicrobial spectrum of these isolates would assist clinicians to select appropriate antimicrobial therapy in order to prevent the dreaded complications of diabetic foot infections.

The study also sought to analyze the influence of patient variables on diabetic foot ulcers. In this study we have made an attempt to correlate $HbA_1C$ levels with the bacteriological profile of diabetic foot infections and the antimicrobial susceptibility pattern.

# AIMS AND OBJECTIVES

1. To study the prevalence of diabetic foot ulcers in various age groups and gender.
2. To isolate and identify the bacterial isolates causing diabetic foot infections.
3. To assess the correlation between Wagner's grade and bacteriological profile.
4. To determine the antibiotic susceptibility pattern of bacterial isolates.
5. To analyze $HbA_1C$ levels in relation with Diabetic Foot Infections, bacteriological profile and antibiotic susceptibility pattern.

## REVIEW OF LITERATURE

Diabetes is one of the oldest metabolic disorders known to mankind. The term "diabetes" is from Ionian Greek, meaning "to pass through".

- The knowledge of Diabetes, dates back to 1550 BC, where descriptions of a polyuric state resembling diabetes mellitus is recorded in the *Ebers Papyrus* by Georg Ebers.
- Association of polyuria with a sweet tasting substance in urine was first reported in Sanskrit literature dating from 5th to 6th century AD at the time of two notable Indian physicians, *Susruta* and *Charaka.*
- The term *"diabetes mellitus*", an allusion to honeyed taste of urine, was first used in late 18th century by *John Rollo* and others, to distinguish it from other polyuric states in which urine was tasteless.
- In 20th century, *Geog Zuelzer* (Germany) and *Nicholas Paulesco* (Romania) isolated active but impure hypoglycaemic extracts from pancreas. (Text of diabetes).
- Late 1970s- dry reagent test strips for self-monitoring of blood glucose were developed.
- 1993- definitive proof was given by the Diabetes Control and Complications Trial that strict glycemic control could slow or prevent the development of diabetic micro vascular complications.[13,14]

**TYPES OF DIABETES**[10, 12]

**Type I Diabetes**: β cell destruction usually leading to absolute insulin deficiency which may be either immune mediated or idiopathic.

**Type II Diabetes**: May be predominantly insulin resistance with relative insulin deficiency or predominantly insulin secretory defect with insulin resistance.

**Type III Diabetes**: Includes genetic defects of B cell function and insulin action, disease of exocrine pancreas and endocrinopathies, drug or chemical induced and infections.

**Type IV Diabetes**: Gestational diabetes.

**Criteria for diagnosing diabetes are**

1) Symptoms of diabetes plus random blood sugar ≥11.1 mmol/lt (200mg/dl) or

2) Fasting plasma glucose ≥ 7 mmol/lt (126mg/dl) or

3) 2 hour plasma glucose ≥ 11.1mmol/lt (200mg/dl) during an oral glucose tolerance test.

**Complications of Diabetes mellitus may be**[3]

**Acute metabolic complications**:

Hypoglycemia, Diabetic ketoacidosis, Hyperosmolar non ketotic coma.

**Late complications**:

Micro vascular (Retinopathy, Neuropathy, Nephropathy),

Macrovascular(Atherosclerosis, Coronary artery disease, Cerebrovascular disease), others(Genitourinary and Gastrointestinal dysfunction) and Diabetic foot ulcers.[10]

## Diabetic Foot Ulcers

Patients with diabetes mellitus may have many serious sequelae. Among them, foot ulcers are most common and may lead to severe complications[50]. Longstanding diabetes often results in peripheral sensory and motor neuropathy, along with foot deformities[8,9]. Peripheral vascular disease and peripheral diabetic neuropathy increase the risk of Diabetic foot ulcers leading to infections and amputations [5, 15]. The longer nerves are more vulnerable hence peripheral foot neuropathy is commonly seen in the foot. These, combined with poorly understood perturbations in host defense mechanisms and wound healing responses, set the stage for diabetic foot ulcers leading to foot infections[64]. Although most of the infections remain superficial, ~25% will spread contiguously from skin to deeper subcutaneous tissues and bone[5, 7].

## Etiopathogenesis of diabetic foot lesions

The diabetic foot lesions have traditionally been considered to result from combination of peripheral neuropathy, vascular disease in leg and infection[25]. More recently, abnormalities of pressure loading on sole of foot and resulting callus formation have been identified as important mediators of the process[7, 14, 18].

## Wagner's classification of diabetic foot lesion

Includes 6 stages of severity[20, 31, 47]:

Grade 0: No obvious ulcer but thick callus, prominent metatarsal head, claw toes or any bony abnormality.

Grade 1: Superficial ulcer, not clinically infected.

Grade 2: Deeper ulcer, often infected, but no bone involvement.

Grade 3: Deep ulcer, abscess formation and bone involvement.

Grade 4: Localised gangrene.

Grade 5: Gangrene of whole foot.

Infections in diabetic patients are mostly polymicrobial in nature [16,19,35]. In acute superficial infections of foot ulcer aerobic gram positive bacteria such as Staphylococcus aureus and Beta Hemolytic Streptococci predominate[21, 26]. In deeply infected chronic ulcers a mixture of aerobic gram positive, aerobic gram negative and anaerobic organism are seen[27, 33].

Staphylococcus aureus was the most common pathogen among the gram positive bacteria isolated among the Diabetic foot ulcers. The pathogenesis of staphylococcal infections is multifactorial. Infection by Staphylococci usually results from a combination of bacterial virulence factors and diminution in host defence[37]. Wound infection can occur following an operative incision, acute traumatic laceration, or chronic pressure induced ulcer, during which bacteria indigenous to the patient or exogenous to the wound overwhelm the systemic and local factors of host resistance[34].

The gram negative comprise mainly of Enterobacteriaceae family such as Escherichia coli, Klebsiella sp, Proteus sp etc. Nonfermentors such as Pseudomonas spp. and Acinetobacter spp. have been isolated as well.

Staphylococci are able to develop resistance quickly and successfully to the antibiotics. This is the consequence of the acquisition and transfer of antibiotic resistance plasmids and the possession of intrinsic resistance mechanisms[44,65]. Methicillin Resistant Staphylococcus aureus (MRSA) is critical global health issue.

Methicillin was a narrow spectrum β-lactam drug developed in the 1950s to tackle c-β-lactamase producing Staphylococcus aureus. Methicillin is no longer manufactured because of its unstable nature and manufacture of a more stable β-lactamase resistant penicllins such as Oxacillin, flucloxacillin and dicloxacillin which are being used. Oxacillin is used as an alternative to Methicillin presently to determine resistance[67].

Methicillin resistance is mediated by mec A gene, which encodes for an alternate Penicillin Binding Protein (PBP) called (PBP) 2. PBPs are enzymes in the cell wall that mediate the formation of cell wall peptidoglycan. PBP 2 exhibits a very low affinity for methicillin and other β-lactam drugs. Thus these penicillin groups of drugs cannot damage cell wall. Cell wall synthesis continues and the bacteria survive. The gene mec-A is carried on a mobile genetic element, the Staphylococcal Cassetee Chromosome mec (SCCmec)[68]. Accessory determinants (fewmA, B, C, fhm B etc) are required for the expression of methicillin resistance without which or alteration in any of these elements decreases the expression of methicillin resistance in spite of the fact that PBP2 is present[44].

External factors affecting methicillin resistance are temperature, PH, osmolarity, light, divalent cations, chelating agents and anaerobiosis, lowering the temperature and increasing the sodium chloride concentration enhances methicillin resistance. These

conditions are routinely employed in the detection of methicillin resistance in clinical isolates[42].

Predisposing factors for MRSA[43]

- Prolonged hospital stay and frequent contact with health care environment
- Close proximity to an infected or colonized patient
- Contact with colonized health care workers.

The emergence of antibiotic resistance in the form of MRSA limits the treatment options available to the clinicians. Detection of MRSA in the early stages of Diabetic foot infections can decrease the morbidity and mortality in these patients[34].

Hemoglobin is the oxygen carrying pigment in RBC. About 90% of Hb(haemoglobin) is HbA (Adult type). 92% of Hb A is made up of major chemical components, 8% of HbA is made up of slightly different minor chemical components. These are Hemoglobin $A_1c$, $A_1b$, $A_1a_1$, and A1a2. Hemoglobin $A_1C$ (HbA1c) is a minor component of Hemoglobin to which glucose is bound. It is called as glycosylated hemoglobin or glycohemoglobin [61]. The more glucose in the blood the more haemoglobin $A_1C$ or Hb $A_1C$ will be present in the blood. Red cells live for 3-12 weeks before they are replaced. $HbA_1C$ level can tell us how high the blood glucose has been on average over the 3-12 weeks period. Normal non diabetic HbA1C is 3.5-5.5%. In diabetes about 6.5% is good [63].

The glycosylated Hb test is an important blood test to diagnose DM/Determine control of DM. There is almost a direct relationship of Foot lesions with increasing Glycosylated Hemoglobin[6,70].

**Review of studies**

The mean age of the patients was, 80.3 years in Delbridge et al study [60], 58 years in Ramani et al study[17], 75.02 years in NA Pathare et al study [31], 58 years in Dipali AC et al study [6] and 43 years in study conducted by C.Anandi et.al. from Tamil Nadu India [1].

D.Vijay et al in 2000 observed a preponderance of male patients showing diabetic foot ulcers (72.5%) compared to female patients (27.5%) The ratio of males to females was 2.6:1[21]. In a study by Dipali AC et al in 2002, 67% of male patients with diabetic foot ulcers were reported against 32.4% of female patients with a ratio of 2.1:1[6]. Anandi et al 2004 observed difference of 65.4% and 54.6% among male and female patients with a ratio of 1.2:1[1]. All the above authors have observed a preponderance of males in their study.

These are various studies done by several investigators on diabetic foot infections and clinical isolates. Mohanty et. al. have studied the Bacterial etiology of soft tissue infection and their Antibiotic susceptibility pattern in 2002. Of the 5,039 pus samples, 2437(48.36%) were culture positive while 1831(33.33%) were culture negative. Among 2437 bacterial isolates 1279 (45.96%) were gram positive cocci. Staphylococcus aureus were 1059 (38.05%). Resistance to Methicillin was detected in 38.56% of Staphylococcus aureus isolates and 31.16% of CONS. [69]

Polymicrobial infection was noted in 64.4% and single etiology in 19.6% in a study conducted by C.Anandi et.al. from Tamil Nadu India[1]. Among the aerobes E.coli 27.7% Pseudomonas species 11.3% and Staphylococcus aureus 13.6% were isolated Clostridium perfringens (31.1%) was the common isolate among the anaerobes. They

concluded that Bacterial culture not only helps in treating infection but also in prevention of developing further complications like Cellulitis and Gangrene [1.]

Polymicrobial Infection was found in 35% of the patients in a prospective study of Diabetic foot ulcers conducted by Ekta Bansal Et.al. Pseudomonas aeruginosa among the gram negative (22%) and Staphylococcus aureus among the gram positive (19%) were the predominantly isolated organisms. While the Candida species was the most predominantly isolated fungus in the study. An average of 1.52 isolates per case was reported in this study. Neuropathy (76%) and peripheral vascular disease 57.28% was a common feature among these patients. Poor glycemic control was found in 67% of patients. [11]

A multicentric clinical trial was conducted by Diane M Ceitron et al; at R.M.Alden Research Laboratories California. Out of the 427 positive cultures 83.8% were polymicrobial. 48% were only aerobes. 43.7% were both aerobes and anaerobes and 1.3% were only anaerobes. The predominant aerobic organisms were Oxacillin susceptible staphylococcus aureus (14.3%), Oxacillin resistant staphylococcus aureus (4.4%), Coagulase Negative Staphylococcus species 15.3%, Streptococcus species 15.5%, Enterococcus species 13.5%, Corynebacterium species 10.1%, and Enterobacteriaceae 12.8%, and Pseudomonas aeruginosa 3.5%. The predominant anaerobes were gram positive cocci [55]

In 654 diabetic patients, 728 pathogens were isolated in study conducted by Vishwanath Et al. Aerobic pathogens were isolated in 437 (66.8%) patients and anaerobic pathogens were isolated in 217(33.2%). Among aerobic pathogens enterobacteriaceae

family (48%), Staphylococcus species (18.2%), Streptococcus species,16.8% and Pseudomonas species 17% were seen frequently. Among anaerobes, Peptostreptococcus species and Clostridium species formed 69.4% [51.]

Gram Negative aerobes 51.4% were most frequently isolated followed by gram positive aerobes and anaerobes (33.3 and 15.3% respectively) in a study conducted by Ravishekar Gadepalli et al from AIIMS New Delhi on Diabetic foot ulcers[58].

In a study conducted by Varaiya et al in Mumbai, Escherichia Coli (40.29 %) and Klebsiella pneumonia 59.70% were isolated.[49]

Uday Kelkar et al in 2004 carried out a comparison study of bacterial yield from the deep tissue samples and swabs in 50 cases with diabetic foot ulcer. The swab samples yielded a total of 150 organisms, comprising 125 aerobes and 25 anaerobes ( average 3.7 organisms per sample).The deep tissue samples yielded a total of 185 organisms comprising of 145 aerobes and 40 anaerobes,. Among the aerobic organisms cultured, Staphylococcus aureus was the most common, followed by Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella species and Enterococcus species.[33]

In a study by Ekta Bansal et al in 2009[11] Proteus sp exhibited 100% sensitivity to Cefaperazone with sulbactum and Ceftriaxone, and amikacin. It showed lowest sensitivity to Amoxicillin (33%). In a study by Vimalin Hena et al in 2010 the proteus isolate was 71% sensitive to Ciprofloxacin 57% to Amikacin[52].

In a study by Ekta Bansal et al in 2009 Pseudomonas showed 100% sensitivity to Imipenem, 94% to Ceftazidime, 83% to Piperacillin63% to Ciprofloxacin. For Amikacin 79% and Gentamicin 33% sensitivity was noticed[11]. In a study by Vimalin Hena et al in 2010 Pseudomonas sp showed 100% sensitivity to Imipenem followed by 83% to Piperacillin, 41% to Ceftazidime and 22% to Ciprofloxacin [52].

In a study by Ekta Bansal et al in 2009 Escherichia coli showed 96% sensitivity to Cefaperazone with sulbactum, 90% to Amikacin, 82% to Ceftazidime and 33% to Ciprofloxacin[11]. In a study by Vimalin Hena et al in 2010 Escherichia coli showed 71% sensitivity to Piperacillin followed by 65% to Ceftazidime. For Amikacin, Gentamicin and Cefotaxime 59% sensitivity was noticed [52].

Anandi et al [1] observed that all the aerobes were sensitive to amikacin and gentamicin except two Pseudomonas spp isolates. All the aerobes were susceptible to Cefotaxime except four Pseudomonas sp isolates which were susceptible to amikacin and gentamicin. Dipali AC et al [6] found that more than 70% of the aerobic gram negative bacilli were sensitive to amino glycosides, amikacin (95.74%) and gentamicin (70.21%). Sensitivity to Cefotaxime was 63.50%. Nema et al found that the gram negative bacilli were most sensitive to amino glycosides and sensitivity to Cefotaxime was 63.12%[43].

Staphylococcus aureus was the most common pathogen isolated from ulcers and almost 50% of the isolates were MRSA in a study conducted by N. Tentolouris et al in patients with infected foot ulcers [65] .

Out of 2314 (37.82%) Staphylococcus aureus strains isolated from diabetic foot ulcers , 992(42.86%) were found to be methicillin resistant in a study conducted at Coimbatore, Tamil Nadu by Muruguan.S, Mani K.R, UmaDevi.[66]

Subedi and Brahmadathan tested 117 Staph aureus strains from patients attending tertiary care centre in western Nepal for susceptibility, 18 (15.4%) were Methicillin resistant. 14 (7 7.8%) of the Methicillin Resistant Strains were Multi Drug Resistant[53].

Dang et. al. anlaysed Diabetic foot ulcers. Gram Positive bacteria were isolated in 84.2% and Staph aureus in (79%) was the commonest single isolate[42.] MRSA was isolated in 30.2% of the patients. They have concluded that there is a need for multi centre study looking into the prevalence of MRSA in diabetic foot ulcer and how it can be reduced in diabetic foot infections.

Kakru et. al. studied 1056 S. aureus from various clinical specimens, among them 312 were from pus samples. 64(35.5%) among 180 samples from outpatients and 42 (31.81%) among 132 samples from inpatients were methicillin resistant. 52% of S. aureus isolates were sensitive to penicillin, 62% to gentamicin, 58% to erythromycin , 53% to co-trimoxazole, 60% to ciprofloxacin, 62% to cefotaxime,6 % to cephalexin, 57% to ampicillin, 73% to amikacin and 100% to vancomycin.[68]

Cerveira et al, did four year study to detect the outcome of MRSA and MSSA infection among lower limb amputation cases. 165 patients underwent lower limb amputation for various causes. Forty-five of these had proven MRSA infection.[67.]

In a study by Nahid Rouhipour 62.9% of patients had poor diabetic control (HbA1C of 8% or higher)[75]. And in **a** study by M.B. Girish et. al the mean glycated hemoglobin was 7.80 ± 0.80 [74]. The patients who underwent amputation presented a significantly higher incidence of ischemic diabetic foot with, HbA1C > 7 [74].

Among 183 diabetic individuals treated at the Johns Hopkins Wound Center. Mean $HbA_1c$ was 8.0%, and there were 2.3 wounds per individual. Of all measures assessed, only $HbA_1c$ was significantly associated with wound-area healing [72].

In Nighat Akbar et al's study Mean value of glycosylated haemoglobin (Hb) was 8.2% (6 - 16.6%).75% of patients showed an $HbA_1c$ level <8.0%; in 13% cases, it was between 8.1 and 10.0%, and in 12% of cases, it was >10%. Data shows that there is almost a direct relationship of foot lesions with increasing Glycated Hb i.e. poorer blood sugar control. All the patients who had an $HbA_1c$ level >10% manifested with various types of foot lesions[73].

In Strhova L et al study in 2006 significant number (65%) of infected ulcers on feet was reported in poorly controlled diabetic patients with $HbA_1C$ above 8%. Infection and osteomyelitis together remains as significant risk factor for amputation. In this study $HbA_1C$ appears to be significant predictor for amputation[62].

As per Wheat et al study the majority of patients with the diabetic foot ulcers had bad control diabetic status ($HbA_1C > 8.5$) but there were no relationship between the bad control diabetic status and the type of pathogen isolated from the ulcers [16.]

In Shaba Tiwari et al study HbA1c was similar in polymicrobial and the mono-microbial infections, (9.9% versus 9.5%; p = 0.1). of diabetic foot patients[71]. Incidence of diabetic foot lesions strongly correlates with the poor glycemic control, which in itself is best manifested by the levels of glycosylated Hb.

## MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology at Coimbatore Medical College Hospital over a period of 1½ years from March 2009 to Sep 2010. Pus and wound swabs were collected from around 100 diabetic patients with foot ulcer attending the Surgery Out-Patient Department of Coimbatore Medical College Hospital. The samples received in the Department of Microbiology were processed for aerobic culture and antibiotic sensitivity testing during the study period. Blood samples were collected to analyze the $HbA_1c$ levels.

**Inclusion criteria:**

- Individuals with Type I and Type II Diabetes mellitus
- Age above 20 years of both sexes.
- Diabetic Foot Ulcer patients including from Grade I to V of Wagner's Classification.

**Exclusion criteria:**

- Patients on antibiotic treatment.
- Foot ulcers of Grade 0.
- Individuals with non diabetic ulcers.

Patients above the age of 20 years, both genders with Diabetes Mellitus were evaluated and the data was collected with the help of questionnaire which comprised of relevant clinical history, clinical examination and laboratory investigations. Clinical examinations involved evaluating the site, nature and extent of the wound.

The Blood Sugar levels were noted. The ulcer type was evaluated using Wagner's classification of diabetic foot ulcers.

**Sample collection**

The surrounding area of the ulcer was cleaned with spirit or povidone iodine and the surface of the wound was washed with sterile normal saline using a sterile cotton swab. Superficial dead tissue and slough was removed with sterile scissors and scalpel. Pus and wound exudates were then obtained with two sterile cotton swabs. One swab was inoculated into Brain heart infusion broth immediately after collection at the bedside for aerobic culture and labeled. Direct smears were made from another swab and stained with Gram stain. The smear was screened for the presence of inflammatory cells and the type of microbial flora.

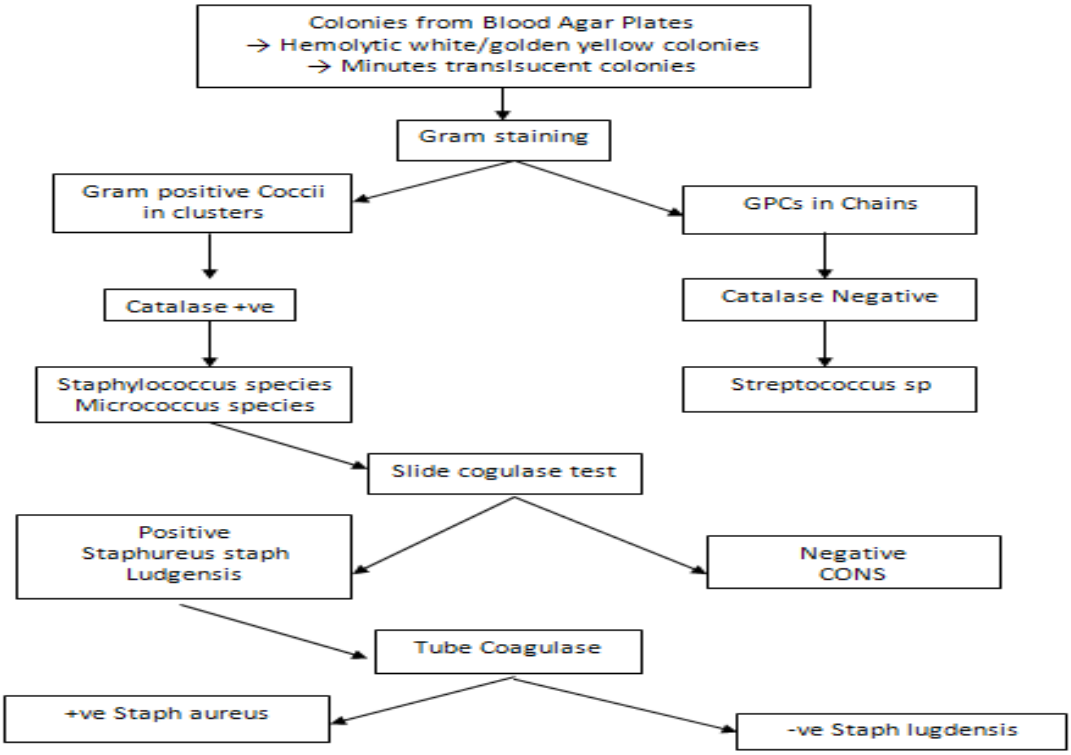
Blood sample was collected under strict aseptic precautions from anterior cubital vein and added to an EDTA containing vacutainer and sent for Blood $HbA_1C$ analysis.

**Characterization of bacterial isolates:**

**Culture of aerobic bacteria**

The inoculated Brain heart infusion broth was incubated overnight at $37^0$C in an incubator. The broth was then sub cultured onto 5% Sheep blood agar, MacConkey agar and nutrient agar plates. The inoculated plates were incubated at $37^0$C overnight. The colonies were examined under magnifying lens for colony morphology, and the isolates were identified using the standard microbiological procedures like Gram staining and biochemical reactions as described in Practical Microbiology of Mackie McCartney 14th edition.[46]

The gram positive cocci are identified as given in the flow chart below:




**Identification of Staphylococci**:

The presence of white opaque colonies on Blood agar plate was further confirmed by examination of gram stained smear. Colonies showing gram positive cocci in clusters were subjected to Catalase test and Coagulase test to identify Staphylococcus aureus.

**Gram staining**

A smear was prepared on a clean grease free glass side. Air dried and heat fixed. The smear was covered with Methyl Violet and allowed to act for about 1 minute. Washed with clean water and the smear was covered with Gram's Iodine for 1 minute. Washed and decolorized with acetone. Washed again immediately and diluted Carbol fuchsin was added on to the slide for 1minute. Washed dried and the stained smear was examined microscopically under oil immersion Objective.

**Catalase Test**

One ml of 3% hydrogen peroxide solution is taken in a small tube. One test colony is picked up using a sterile glass rod and introduced into the solution. Vigorous effervescence indicates catalase activity and was taken as positive.

Positive control – Staphylococcus aureus.

Negative control – Enterococcus fecalis.

**Coagulase Test**

Citrated human plasma was used for the test.

**Slide Coagulase test**

1-2 colonies of staphylococcus were emulsified in a drop of normal saline on a grease free glass slide to form a smooth milky suspension. Similar suspensions were made for positive and negative controls. Then a drop of undiluted plasma was added to all the three suspension. Coarse clumping of the organisms visible to the naked eye within 10 seconds was taken positive. If the test was negative or showed slow reaction, tube coagulase was done.

**Tube Coagulase test**

1 ml of 1:6 dilution of plasma in saline was taken in a small tube. 1-2 colonies were emulsified in the tube of diluted plasma. Positive and Negative Coagulase controls and plasma controls were set up. The tubes were incubated at $37^0$C for 4 hours. The tubes were examined at 1, 2 and 4 hours interval for clot formation.

**Antibiotic Susceptibility testing**

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method as per CLSI guidelines. The isolates were grown in peptone water by incubating at $37^0$ C till the turbidity matched that of 0.5 MacFarland standard .They were then lawn cultured onto Mueller Hinton agar plate and commercial antibiotic discs [Penicillin(10U), Erythromycin(15µg), Ampicillin (10µg), Amoxyclav (30µg), Gentamicin(10µg), Amikacin(30µg), Linezolid (10µg), Cefotaxime (30µg), Cephalexin(30 µg), Ciprofloxacin(5µg), Vancomycin(30µg), Co-trimoxazole (25µg)] procured from Hi media, Mumbai were placed on the surface. The plates were incubated overnight at $37^0$C and the zones of inhibition were measured and interpreted according to the charts provided by the manufacturers.[57] Staphylococcus aureus ATCC 25923 was used as a control for the susceptibility testing.

Staphylococcus aureus isolates were subjected to Methicillin susceptibility testing by Kirby-Bauer disc diffusion method using Oxacillin (1 µg) disc. 1 to 2 Staphylococcal colonies were suspended in 0.5 ml of sterile normal saline and the turbidity matched to 0.5 McFarland. Using sterile cotton swab the broth culture was uniformly streaked on to Mueller Hinton agar with 2% Sodium Chloride Plate. Oxacillin (1 µg) disc was placed on the plates were incubated at $37^0$C aerobically for 24 hrs and the zone of inhibition was measured. Staphylococcus aureus ATCC 43300 was used as a control for methicillin resistance.

The gram negative bacilli is identified based on the colony morphology on MacConkey agar plate and biochemical reactions as given in the table below:

IDENTIFICATION OF ENTEROBACTERIACEAE

Lactose and non lactose fermenting colonies present on Mac Conkey agar were subjected to gram staining & biochemical reactions as mentioned below for confirming them to be from family enterobacteriaceae.

| Bacterial Isolates | Catalase | Oxidase | Indole | MR | VP | Citrate | Urease | TSI | Glu | Lac | Suc | Malt | Mann |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| E.Coli | + | - | + | + | - | NU | NH | A/A+- | + | + | +/- | + | + |
| Klebsiella | + | - | - | - | + | U | H | A/A+- | + | + | + | + | + |
| P.mirabilis | + | - | - | + | _ | U | H | K/A++ | + | +/_ | +/_ | _ | + |
| P.vulgaris | + | - | + | + | _ | U | H | K/A++ | + | _ | + | + | _ |
| C.freundii | + | - | - | + | _ | U | NH | K/A++ | + | _ | + | + | _ |
| E.cloacae | + | - | - | - | + | U/NU | H/NH | A/A+- | + | + | + | + | + |

U- Utilised , NU – Not utilised , H- Hydrolysed, NH - Not Hydrolysed ,MR Methyl Red, VP- Voges Proskauer

TSI- Triple Sugar Iron, A-Acid, K – Alkaline.

The Non-fermenters are identified based on the non-lactose fermenting colonies on MacConkey agar plate and the biochemical reactions as given in the table below:

## Biochemical reactions for Identification of Non-fermenters

| Organism | Pseudomonas spp | Acinetobacter spp |
|---|---|---|
| Catalase | P | P |
| Oxidase | P | N |
| O/F | O | NF |
| Indole | N | N |
| MR | N | N |
| VP | N | N |
| Citrate | Utilized | - |
| Urease | V | - |
| TSI | K/K | K/K |
| MM | NF/M | NF/NM |

MM-Mannitol motility VP- Voges Proskauer V-variable

O/F- Oxidation/Fermentative MR- Methyl red TSI- Triple Sugar Iron

N- Negative P- Positive NF- Not fermented

## ANTIBIOTIC SENSITIVITY TESTING

Antimicrobial susceptibility testing was performed by Kirby Bauer disk diffusion method. Commercially available Mueller Hinton agar culture medium and antibiotic discs (Himedia) were used.

### Inoculum preparation

Using sterile wire loop 3-4 well isolated colonies of similar appearance from primary culture plate were inoculated into 2-3 ml of normal saline and subsequently emulsified. The turbidity was adjusted so as to correspond to the 0.5 Mcfarland standards.

### Preparation of 0.5 McFarland standard.

0.5 ml of aliquot of 0.08mol/L (1.175% w/v $BaCl_2$ 2H20) is added to 99.5 ml of 0.18 mol/l $H_2SO_4$ (1%V/V) with constant stirring to maintain a suspension.

### Inoculation of test plates

After adjusting the turbidity of the inoculum suspension, sterile cotton swab dipped into the suspension. Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of suspension. The surface of the Muller Hinton agar plate was streaked with the swab evenly over in three directions. Using sterile forceps the appropriate antimicrobial discs were placed over the inoculated plate and not closer than about 24 mm from disc to disc. Then the plates were incubated at $37^{o}$c for 18-24 hrs.

The commercial discs used were procured from Hi media lab ltd. Ampicillin (10μg), Gentamicin (10μg), Amikacin (30μg), Ciprofloxacin (5μg), Cotrimoxazole (25μg), Cefotaxime (30μg). Cephalexin (30 μg), Ceftriaxone (30μg), Cefaperazone with sulbactum (75 μg), Piperacillin with tazobactum (100 μg), Ofloxacin (5 μg), Ceftazidime(30 μg) & Meropenem (10 μg).

**Interpretation of zone sizes**

The zones of inhibition were measured and interpreted according to the charts provided by the manufacturers.[57]

## RESULTS

The present study was carried out in the Department of Microbiology, CMC from March 2009 to Sep 2010 to look for the pattern of growth of aerobic organisms and their antibacterial susceptibility pattern in diabetic foot infections. $HbA_1C$ levels are also determined in the study. The following Tables and Figures illustrate the results in detail. The results obtained were analyzed.

**TABLE 1: AGE AND SEX DISTRIBUTION OF DIABETIC FOOT ULCER CASES**

| Age in years | Males | Females |
|---|---|---|
| 21-30 | 3 | 1 |
| 31-40 | 4 | 1 |
| 41-50 | 14 | 4 |
| 51-60 | 26 | 11 |
| 61-70 | 16 | 12 |
| 71-80 | 7 | 1 |
| Total | **70** | **30** |

**Chart 1: AGE AND SEXWISE DISTRIBUTION OF DFI CASES**

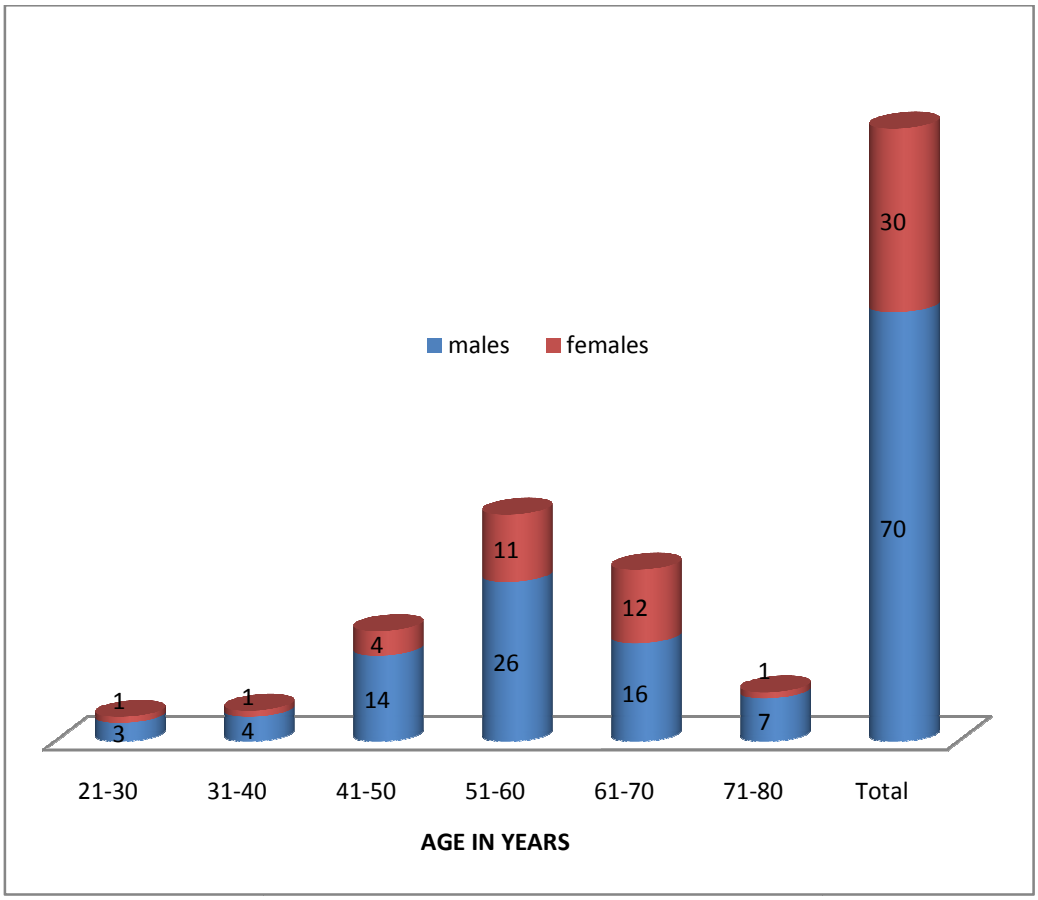
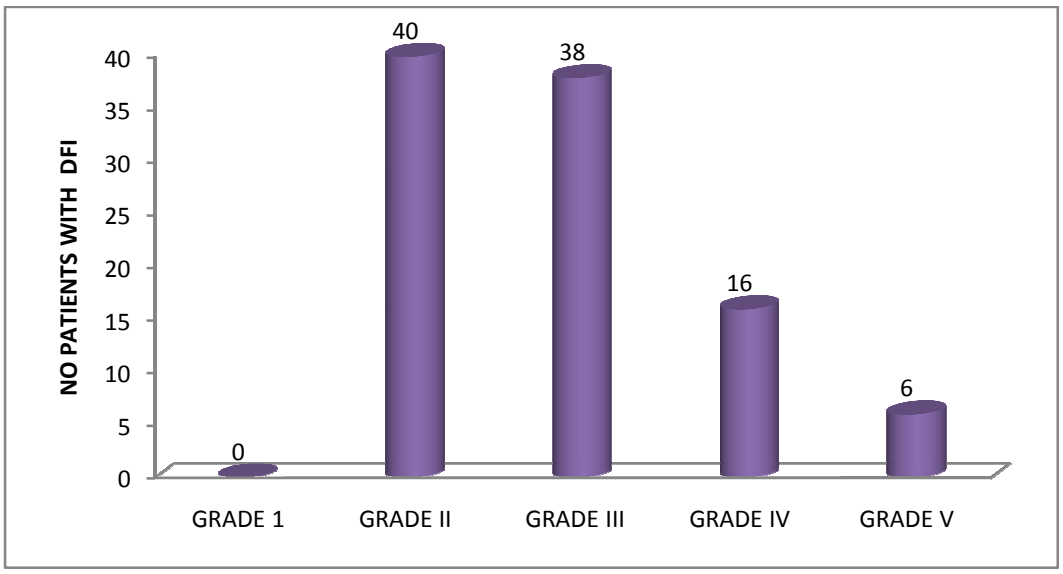



Table & Chart 1 shows the distribution of age and sex among the cases of DFI. Of The 100 cases studied, most of the patients belonged to the 5$^{th}$ and 6$^{th}$ decades of life (37%) and (28%) respectively. Males were more affected compared to females with a ratio of 2.3:1.

**TABLE 2: DISTRIBUTION OF ULCERS ACCORDING TO WAGNER'S CLASSIFICATION**

| Wagner's Grade | GRADE I | GRADE II | GRADE III | GRADE IV | GRADE V |
|---|---|---|---|---|---|
| **No. of Patients with Diabetic foot ulcers** | 0 | 40 | 38 | 16 | 6 |

**Chart2: DISTRIBUTION OF ULCERS ACCORDING TO WAGNER'S CLASSIFICATION**




Distribution of Ulcers According to Wagner's Classification are listed in Table and Chart 2. Maximum number of patients with Diabetic Foot Ulcers were seen in **Wagner's** Grade II (40 nos), followed by 38 DFI patients in Wagner's Grade III.

**TABLE 3: DISTRIBUTION OF BACTERIAL ISOLATES IN CORRELATION WITH WAGNER'S GRADE**

| | GRADE I | GRADE II | GRADE III | GRADE IV | GRADE V |
|---|---|---|---|---|---|
| No of patients with Diabetic foot ulcers | 0 | 40 | 38 | 16 | 6 |
| No of organisms isolated (Aerobes) | 0 | 40 | 40 | 35 | 10 |
| Average number of micro organisms /sample | 0 | 1 | 1.05 | 2.18 | 1.66 |

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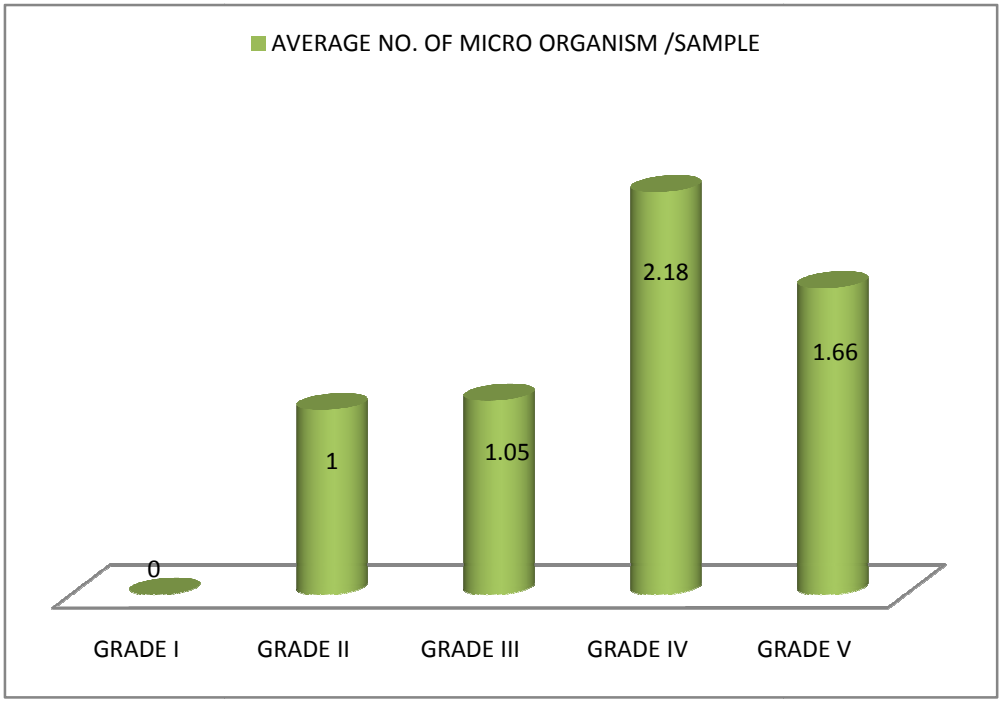
**CHART 3: DISTRIBUTION OF AVERAGE NO. OF BACTERIAL ISOLATES PER SAMPLE IN CORRELATION WITH WAGNER'S GRADE**




• Average no of aerobes per sample was found to be maximum in Grade 4 ulcers (2.18).

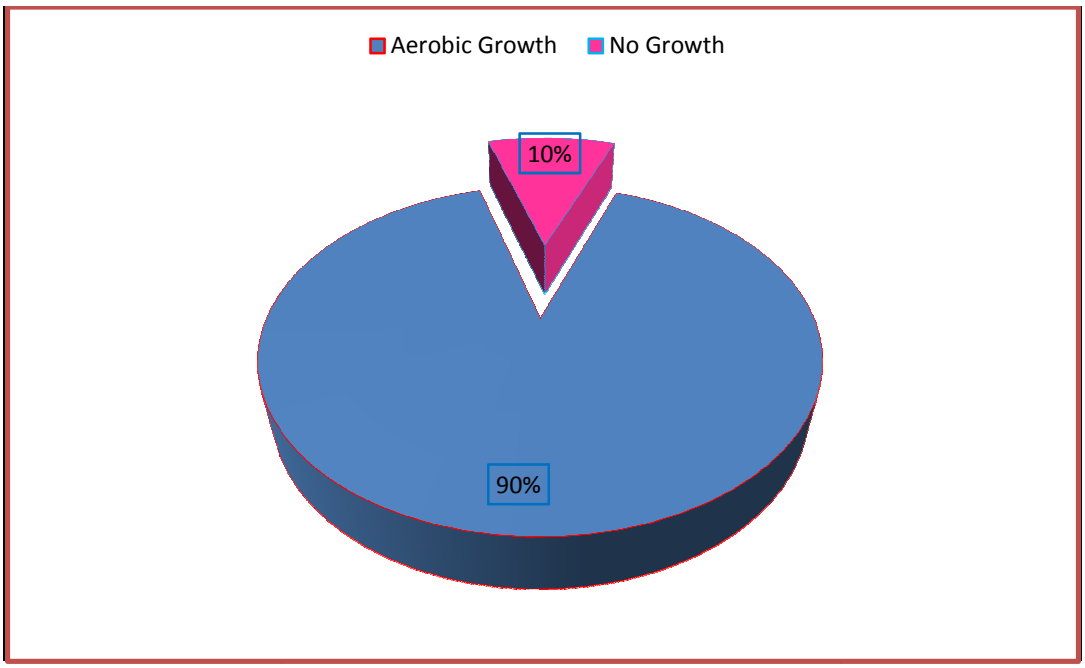
• The average number of microorganism /sample is decreasing as the Wagner's grade Decreases.

*The number of isolates are more than the number of samples and the average number of microorganism /sample is more than one because of poly microbial growth yield.

**TABLE 4 : AEROBIC GROWTH DISTRIBUTION**

| | |
|---|---|
| Aerobic Growth | 90 |
| No Growth | 10 |

**CHART 4 : AEROBIC GROWTH DISTRIBUTIONS**

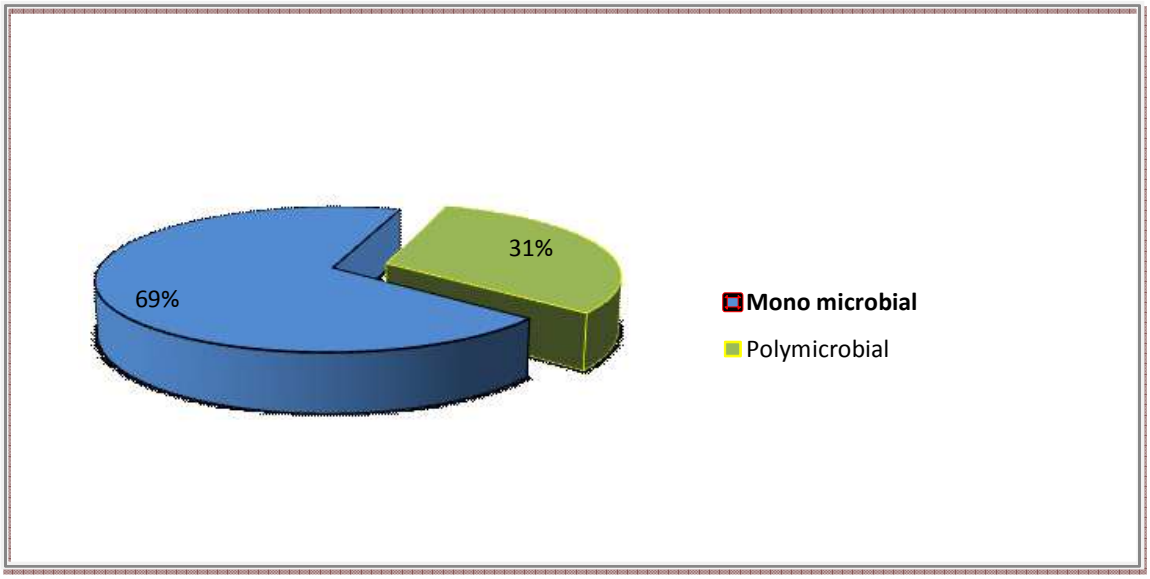



Hundred pus samples were collected from foot ulcers of diabetic patients and assessed for the growth of aerobic organisms and listed in Table 4 & Chart 4. Out of the hundred samples 90 yielded aerobic bacterial growth and10 samples did not yield any growth.

**Table-5: POLYMICROBIAL AND MONO-MICROBIAL GROWTH DISTRIBUTION**

| Growth pattern | Number | Percentage |
|---|---|---|
| Mono microbial | 62 | 69% |
| Polymicrobial | 28 | 31% |

**CHART-5: POLYMICROBIAL AND MONO-MICROBIAL GROWTH DISTRIBUTION**




The pattern of growth is listed in Table5 & Chart 5 .Out of the 90 culture positive samples mono microbial growth was found in 62 samples and 28 samples yielded polymicrobial growth with a percentage of 69 and 31 respectively.

**TABLE 6: DISTRIBUTION OF BACTERIAL ISOLATES.**

| **GRAM POSITIVE ISOLATES** | **No. of aerobes(n=125)** | **Percentage (%)** |
|---|---|---|
| Staphylococcus aureus | **23** | **18.4** |
| Coagulase negative Staphylococcus( CONS) | **8** | **6.4** |
| Enterococcus fecalis | **1** | **0.8** |
| **GRAM NEGATIVE ISOLATES** | | |
| Proteus sp | **29** | **23.2** |
| Pseudomonas aeruginosa | **20** | **16** |
| E.coli | **21** | **16.8** |
| Klebsiella sp | **11** | **8.8** |
| Enterobacter spp | **5** | **4** |
| Citrobacter freundii | **4** | **3.2** |
| Acinetobacter spp | **3** | **2.4** |

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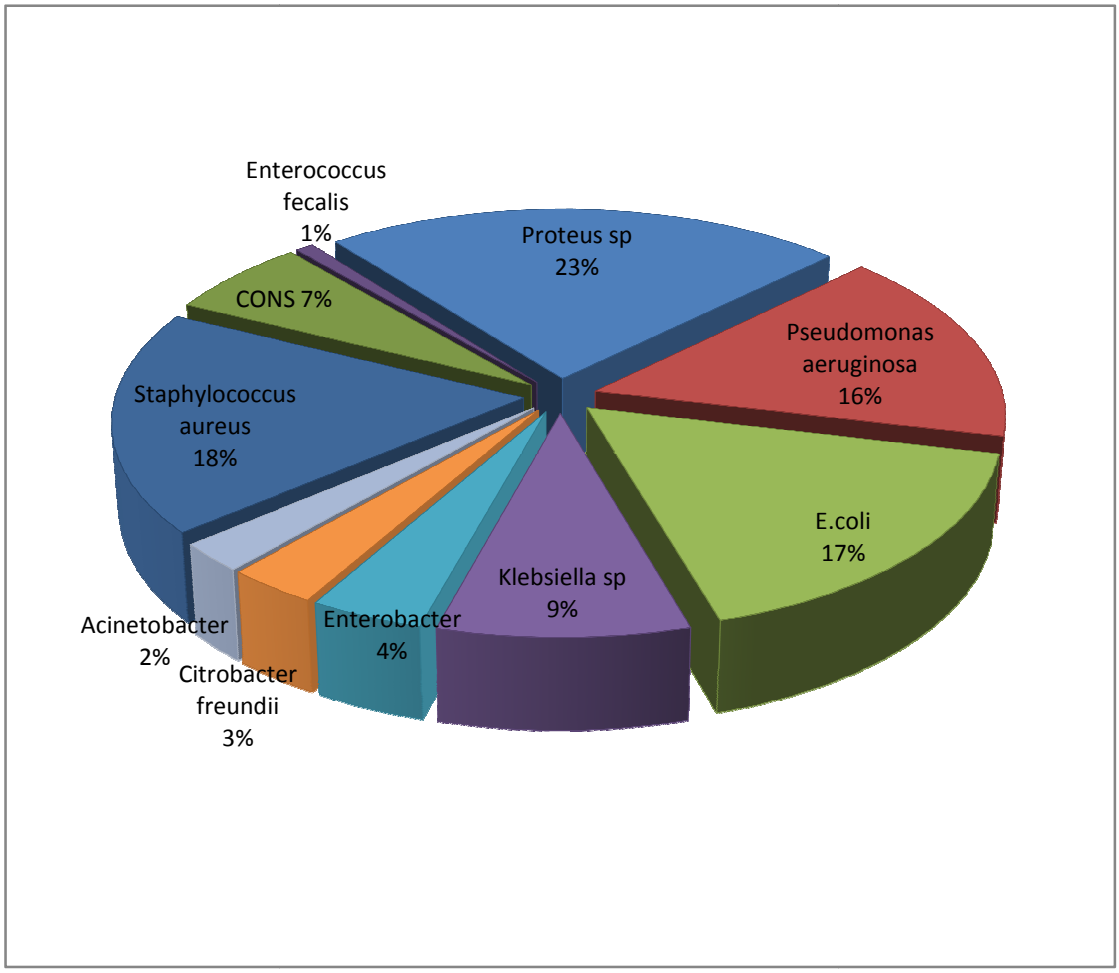
**Chart6: DISTRIBUTION OF BACTERIAL ISOLATES.**




Distribution of aerobic bacterial isolates are listed as per Table 6 & Chart 6.

- Among Gram positive aerobes, Staphylococcus aureus was the predominant Isolate (18.4%).
- Among Gram negative aerobes, Proteus spp was the most common isolate (23.2%) followed by E.Coli 16.8% and Pseudomonas 16%. Acinetobacter species was the least common isolate (2.4%).

**TABLE 7: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM POSITIVE COCCI**

| Antibiotics | Number of Susceptible Isolates | | | | | |
|---|---|---|---|---|---|---|
| | Staphylococcus aureus(23) No. | % | CONS(6) | | Enterococcus fecalis(1) | |
| Ak | 16 | 69.5% | 4 | 66.6% | - | - |
| G | 10 | 43.4% | 2 | 33% | - | - |
| Am | 6 | 26.08% | 2 | 33% | 0 | 0 |
| Cip | 11 | 47.8% | 3 | 50% | 0 | 0 |
| Of | 12 | 52.2% | 3 | 50% | 0 | 0 |
| Cp | 1 | 4.3% | 1 | 16.6% | 0 | 0 |
| Ce | 6 | 26.08% | 2 | 33.3% | 0 | 0 |
| E | 8 | 34.7% | 1 | 16.6% | 0 | 0 |
| Do | 6 | 26.08% | 2 | 33% | - | - |
| Ac | 12 | 52.2% | 4 | 66% | 0 | 0 |
| Co | 2 | 8.6% | 1 | 16.6% | 0 | 0 |
| Lz | 23 | 100% | 6 | 100% | 1 | - |
| Van | 23 | 100% | 6 | 100% | 1 | - |

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**CHART 7: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM POSITIVE COCCI**

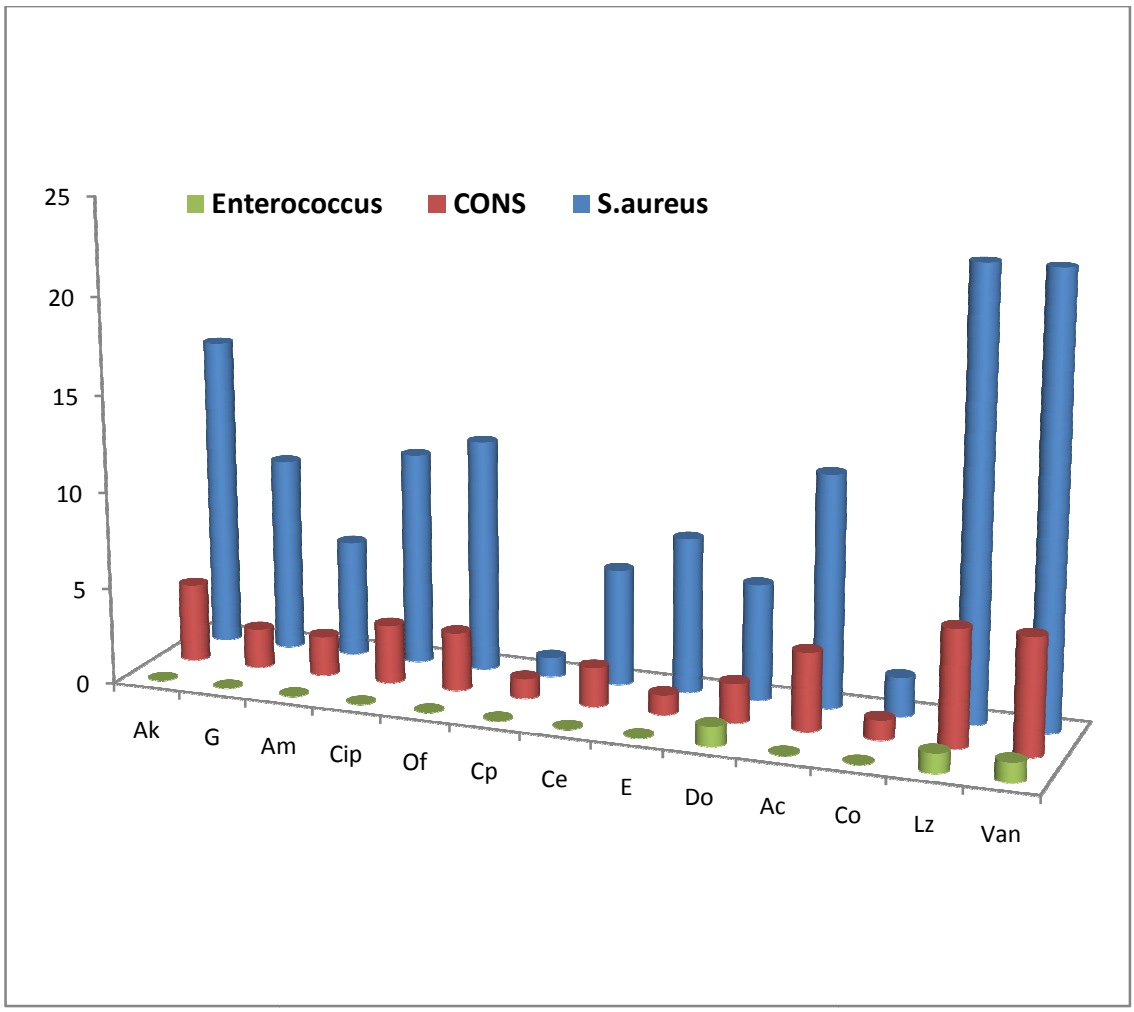



Table & Chart 7 shows the Antimicrobial Susceptibility Pattern of Gram Positive Cocci. Staphylococcus aureus showing sensitivity of 26% to Cefotaxime, 52% to Ofloxacin and Amoxyclav & 47.8% to Ciprofloxacin. CONS isolates are 66% sensitive to Amikacin. And all these isolates show 100% sensitivity to Vancomycin and Linezolid.

TABLE 8: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE ISOLATES

| AEROBES | Cot | Amp | Ak | G | Cip | Of | Caz | Ctx | Ci | Ac | Cfs | Pit | Mrp |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Proteus(29)** | **10** | **4** | **13** | **11** | **15** | **20** | **13** | **22** | **24** | **8.4** | **24** | **29** | **-** |
| | **34%** | **15%** | **46%** | **34%** | **53%** | **69%** | **46%** | **76%** | **84%** | **26%** | **84%** | **100%** | |
| **Pseudomonas spp (20)** | **-** | **0** | **2** | **2** | **9** | **9** | **10** | **9** | **8** | **11** | **14** | **19** | **19** |
| | | | **10.5%** | **10.5%** | **47%** | **47%** | **52%** | **47%** | **42%** | **57%** | **68%** | **94%** | **100%** |
| **E.coli (21)** | **2** | **11** | **17** | **12** | **10** | **7** | **9** | **16** | **15** | **6** | **21** | **21** | **--** |
| | **11%** | **52%** | **82%** | **58%** | **47%** | **42%** | **47%** | **76%** | **70%** | **29%** | **100%** | **100%** | |
| **Klebsiella Pneumonia(11)** | **1** | **3** | **6** | **6** | **11** | **10** | **7** | **7** | **7** | **4** | **4** | **11** | **-** |
| | **12.50%** | **25%** | **50%** | **50%** | **100%** | **87.5%** | **62.5%** | **62.5%** | **62.5%** | **37.5%** | **87.5%** | **100%** | |
| **Enterobacter Species(5)** | **2** | **2** | **3** | **2** | **5** | **5** | **3** | **4** | **4** | **3** | **5** | **5** | **-** |
| | **40%** | **40%** | **60%** | **40%** | **100%** | **100%** | **60%** | **80%** | **80%** | **60%** | **100%** | **100%** | |
| **Citrobacter freundii(4)** | **2** | **3** | **3** | **3** | **3** | **3** | **3** | **4** | **4** | **3** | **4** | **4** | **-** |
| | **50%** | **75%** | **75%** | **75%** | **75%** | **75%** | **75%** | **100%** | **100%** | **75%** | **100%** | **100%** | |
| **Acinetobacter sp.(3)** | **1** | **1** | **3** | **2** | **3** | **3** | **2** | **2** | **2** | **2** | **3** | **3** | **-** |
| | **33.30%** | **33.30%** | **100%** | **50%** | **100%** | **100%** | **66%** | **66%** | **66%** | **66%** | **100%** | **100%** | |

- Proteus sp showed 100% sensitivity to Piperacillin with tazobactum, 84% to Cefaperazone with sulbactum and Ceftriaxone,
  76%. sensitivity to Cefotaxime, 53% to Ciprofloxacin and 46% to amikacin.
- Pseudomonas sp showed 100% sensitivity to Meropenum followed by 94% to Piperacillin with tazobactum,
  68% to Cefaperazone with sulbactum, 52% to Ceftazidime, and 47% to Ciprofloxacin. Amikacin and Gentamicin showed 10.5% sensitivity.
- Escherichia coli showed highest sensitivity to Piperacillin with tazobactum and Cefaperazone with sulbactum, 82% to amikacin, 76% to Cefotaxime and Ceftriaxone (70%) and 47% to Ceftazidime.

CHART 8: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE ISOLATES

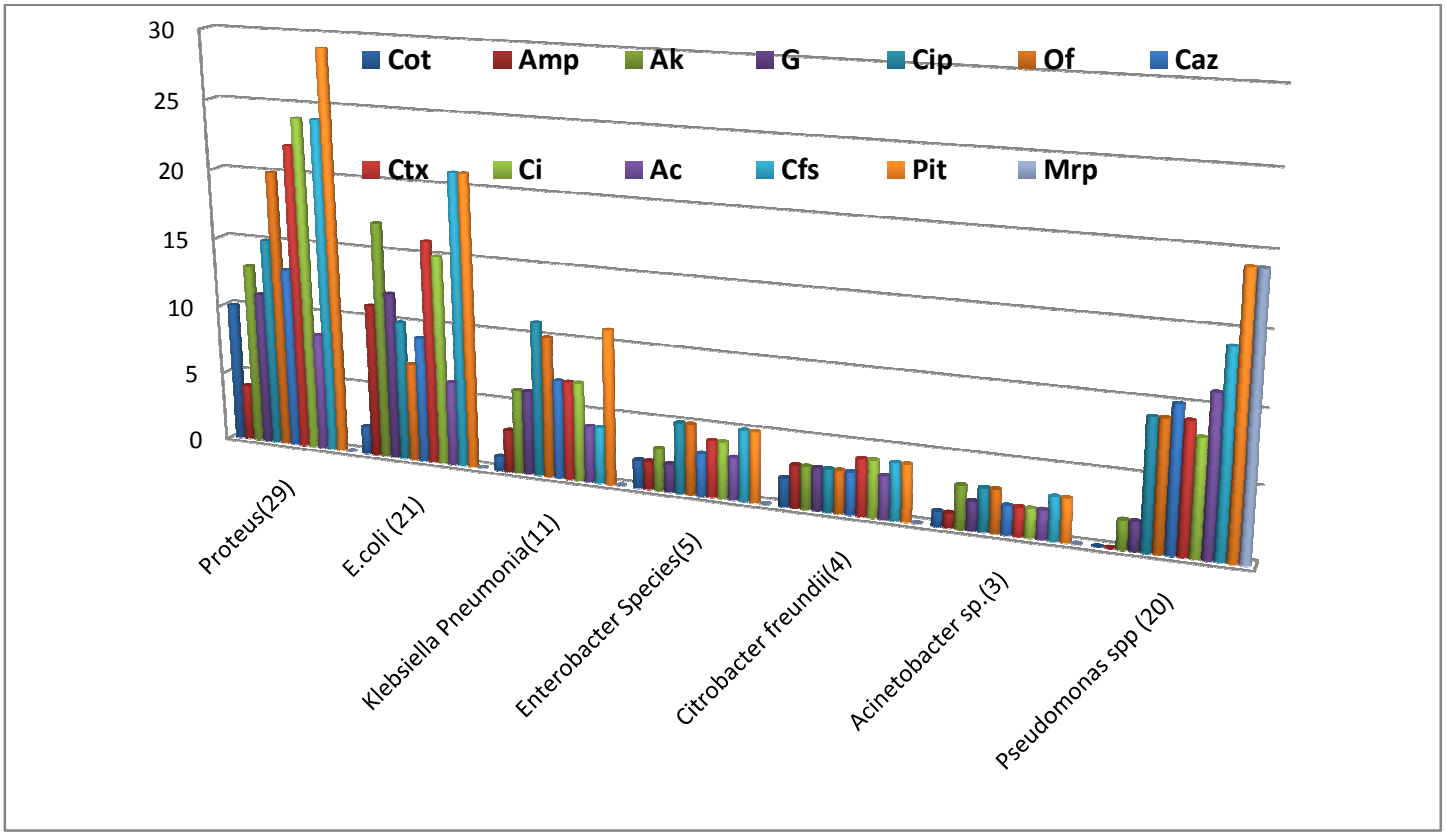
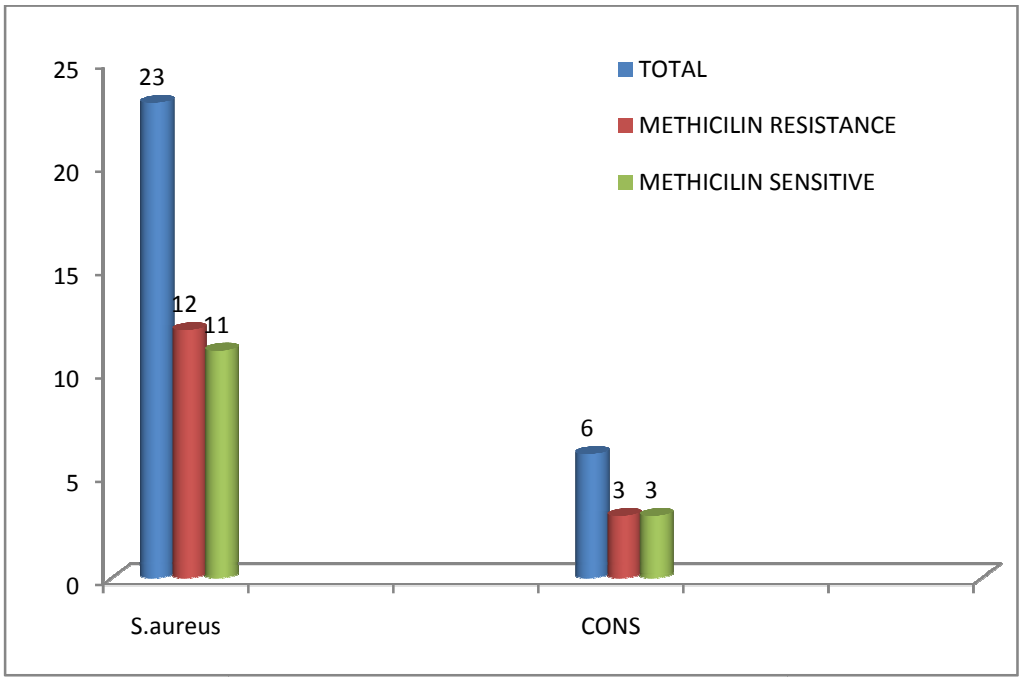


**Table 9: DISTRIBUTION OF METHICILLIN SENSITIVE & METHICILLIN RESISTANT STAPHYLOCOCCI**

| | Total No Isolated | Methicillin Resistant Staphylococci | Methicillin Sensitive Staphylococci |
|---|---|---|---|
| Staphylococcus aureus | 23 | 12 | 11 |
| CONS | 6 | 3 | 3 |

**CHART 9: DISTRIBUTION OF METHICILLIN SENSITIVE & METHICILLIN RESISTANT STAPHYLOCOCCI**

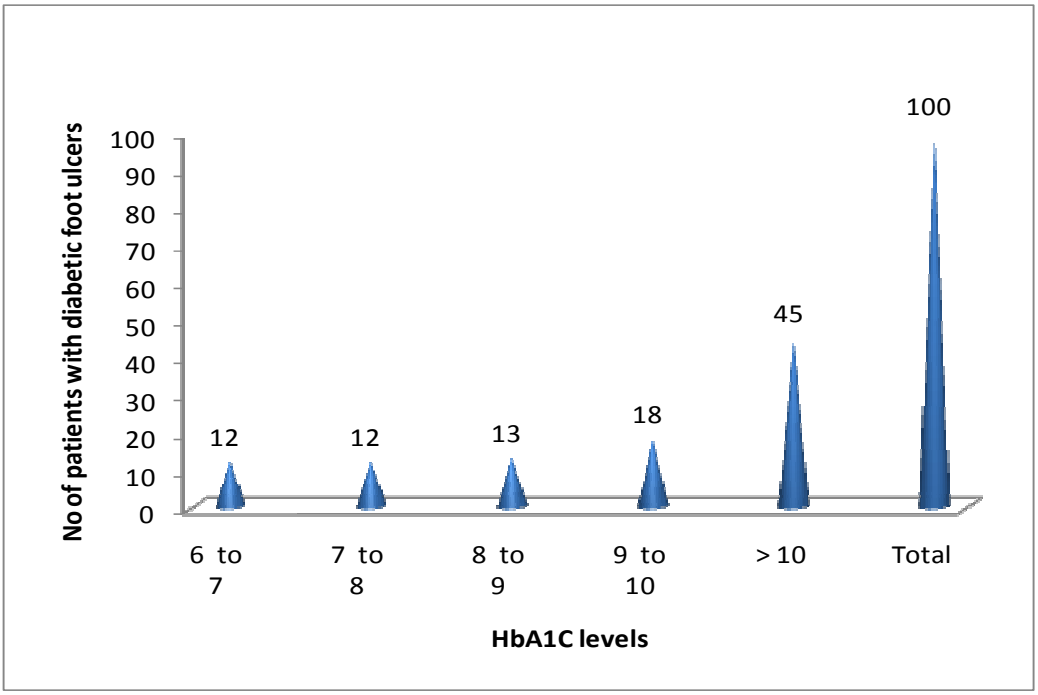



As evident from Table & chart 9, antimicrobial susceptibility testing revealed the methicillin resistance. Among 23 Staphylococcus aureus isolates 12 were Methicillin resistant (55%) and 11 were methicillin sensitive. CONS isolates exhibited 50% Methicillin resistance.

## TABLE 10: CORRELATION BETWEEN $HBA_1C$ LEVELS AND DIABETIC FOOT ULCERS

| $HbA_1C$ Levels | No of patients with diabetic foot ulcers |
|---|---|
| 6 to 7 | 12% |
| 7 to 8 | 12% |
| 8 to 9 | 13% |
| 9 to 10 | 18% |
| > 10 | 45% |
| Total | 100 |

## CHART 10: CORRELATION BETWEEN HBA$_1$C LEVELS AND DIABETIC FOOT ULCERS




- Maximum No. of DFI patients had HbA$_1$C levels more than 10.
- The number of patients having HbA$_1$C levels above 8 was 76%.

## Table`11: CORRELATION BETWEEN $HBA_1C$ LEVELS AND WAGNER'S GRADES

| | Wagner's Grade I | Wagner's Grade II | Wagner's Grade III | Wagner's Grade IV | Wagner's Grade V | Total |
|---|---|---|---|---|---|---|
| 6 to 7 | 0 | 6 | 6 | 0 | 0 | 12 |
| 7 to 8 | 0 | 6 | 3 | 1 | 2 | 12 |
| 8 to 9 | 0 | 6 | 4 | 2 | 1 | 13 |
| 9 to 10 | 0 | 4 | 8 | 4 | 2 | 18 |
| > 10 | 0 | 18 | 17 | 9 | 1 | 45 |
| Total | 0 | 40 | 38 | 16 | 6 | 100 |

**Chart`11: CORRELATION BETWEEN HBA$_1$C LEVELS AND WAGNER'S GRADES**

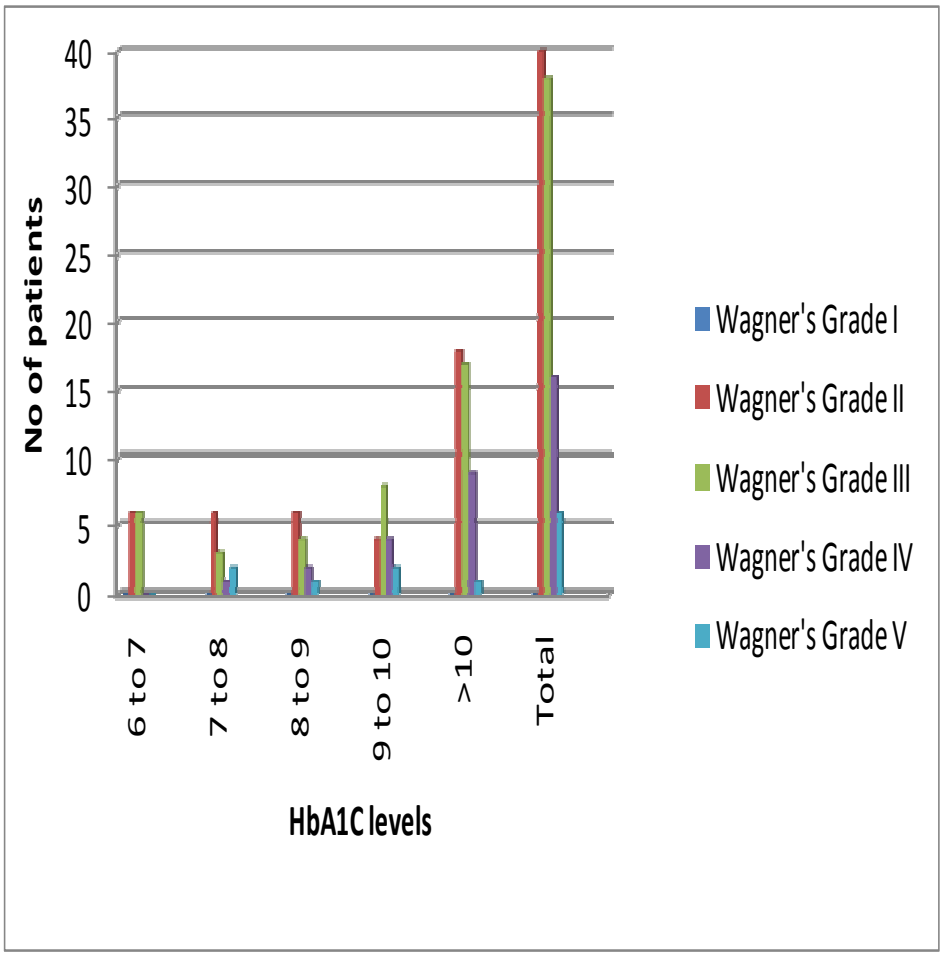



Table 11 & Chart 11 Shows The Correlation Between HbA$_1$c Levels And Wagner's Grades.

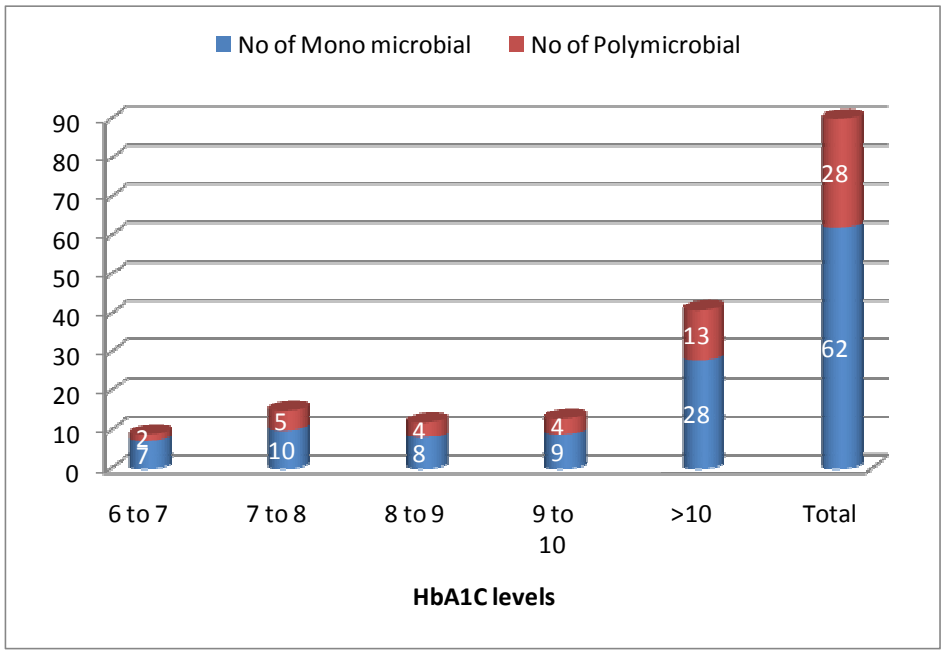
Maximum no of cases (45) were recorded with HbA$_1$C levels of >10

**TABLE12: CORRELATION BETWEEN $HbA_1C$ LEVELS AND MICROBIAL GROWTH.**

| $HbA_1C$ Levels | No of Mono microbial | No of Polymicrobial |
|---|---|---|
| 6 to 7 | 7 | 2 |
| 7 to 8 | 10 | 5 |
| 8 to 9 | 8 | 4 |
| 9 to 10 | 9 | 4 |
| > 10 | 28 | 13 |
| Total | 62 | 28 |

**PTO**

**CHART12: CORRELATION BETWEEN $HbA_1C$ LEVELS AND MICROBIAL GROWTH**

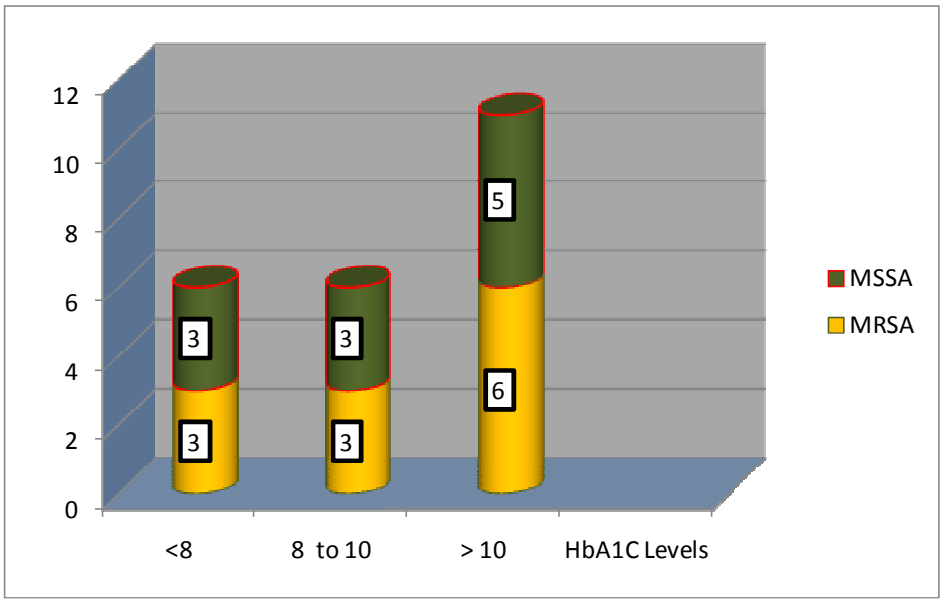



The total distribution of Mono microbial and polymicrobial growth was 62 and 28 respectively with the maximum number of growth recorded in $HbA_1C$ levels of more than 10.

**TABLE 13: CORRELATION BETWEEN $HBA_1C$ LEVELS AND MRSA**

| $HbA_1C$ Levels | MRSA | MSSA |
|---|---|---|
| <8 | 3 | 3 |
| 8 to 10 | 3 | 3 |
| > 10 | 6 | 5 |

**CHART 13: CORRELATION BETWEEN $HBA_1C$ LEVELS AND MRSA**



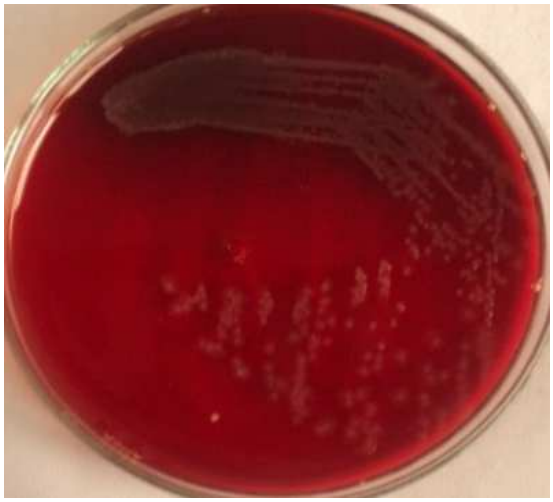

The Table 13 & Chart 13 reveals the correlation between $HbA_1C$ levels and MRSA. Of the 23 Staphylococcal isolates MRSA constitutes 50% in $HbA_1$**c** Levels of <8 and 8 to 10 and 55% in $HbA_1$**c** Levels of > 10

Staphylococcus aureus on  Blood agar

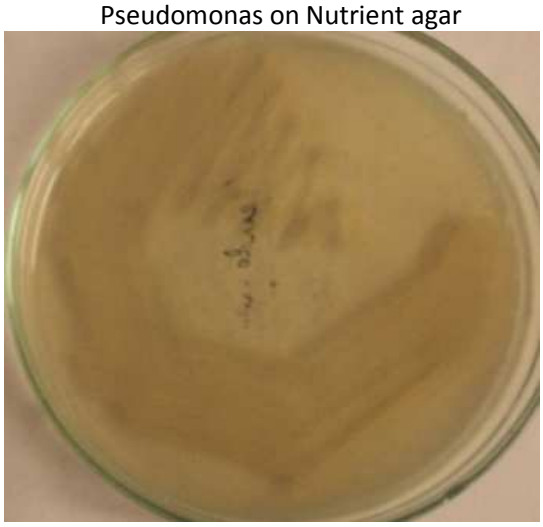
Staphylococcus aureus on Nutrient agar


Tube Coagulase test

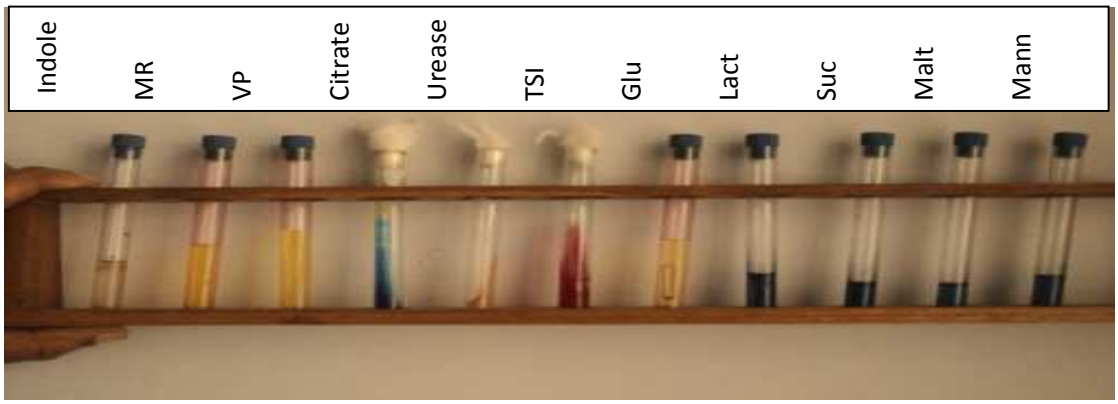
Pseudomonas on Blood agar



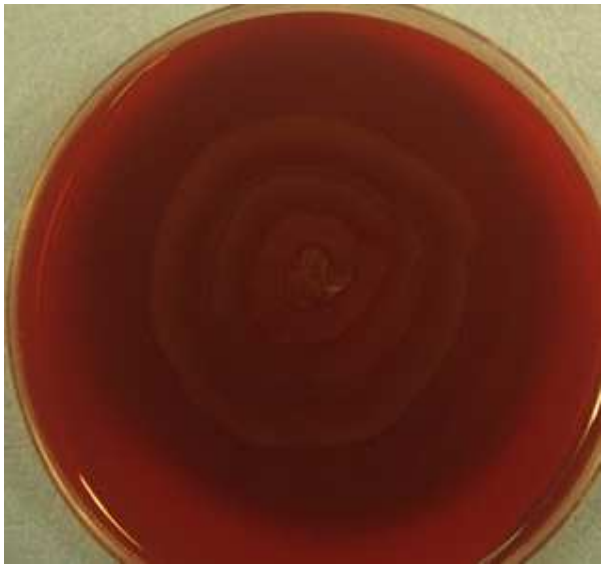
Pseudomonas on Nutrient agar



Biochemical Reactions of Pseudomonas

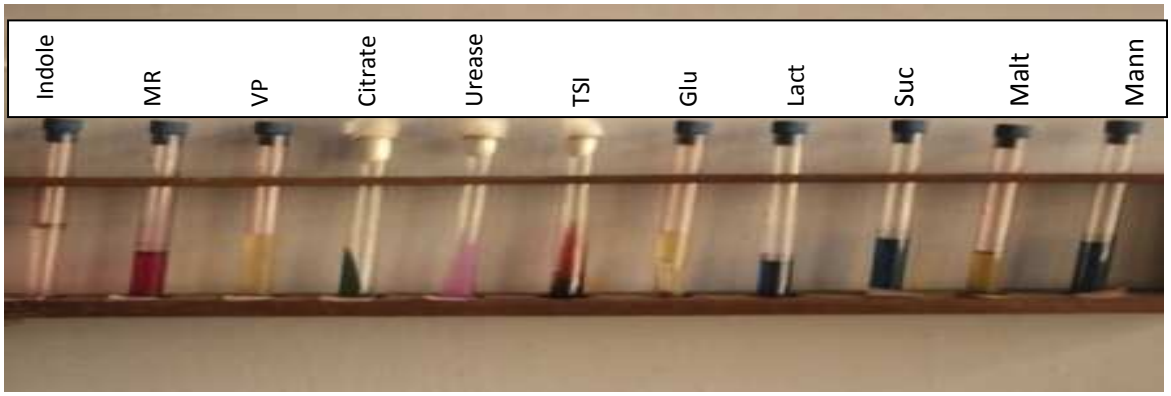


Proteus on Blood agar



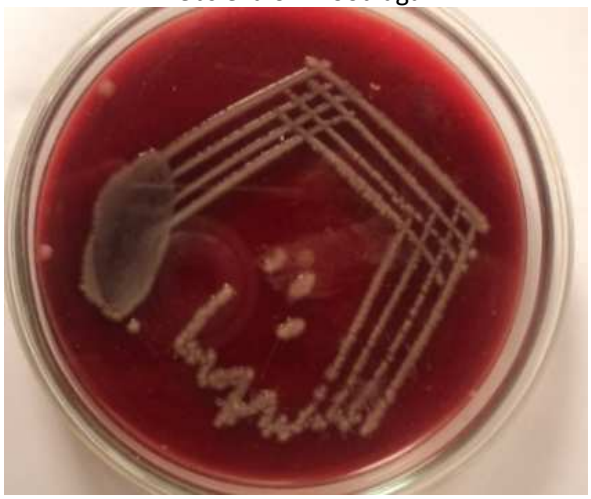
Proteus on Nutrient Agar

BioChemical Reactions of Proteus

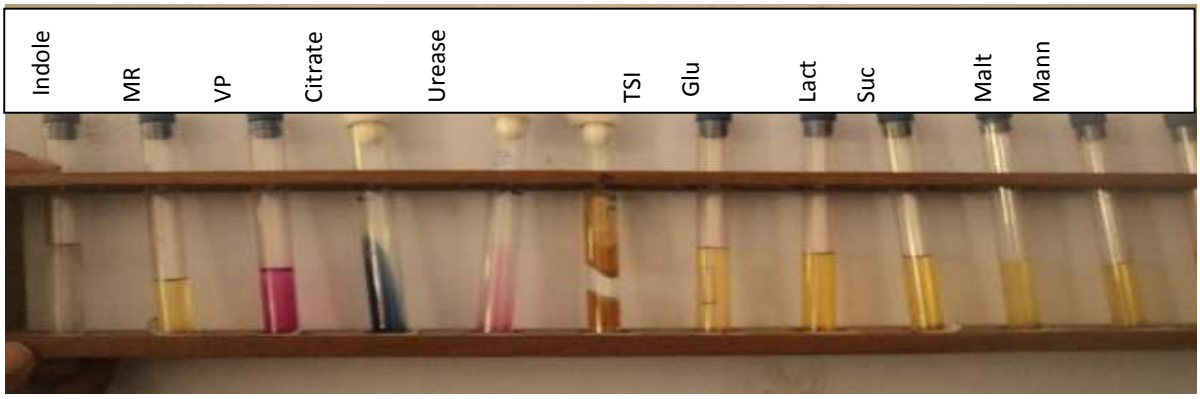


Klebsiella on MacConkey agar

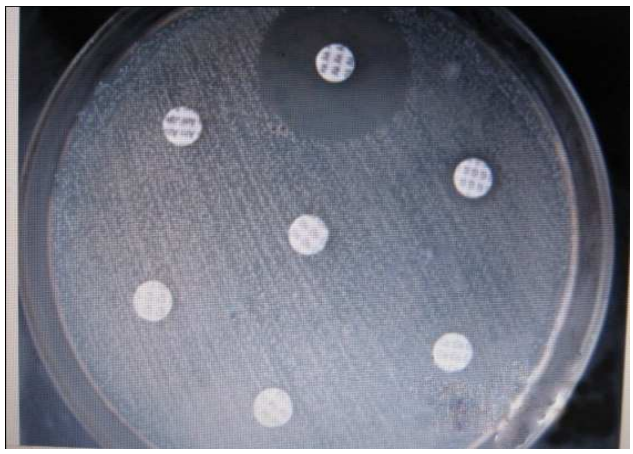
Klebsiella on Blood agar



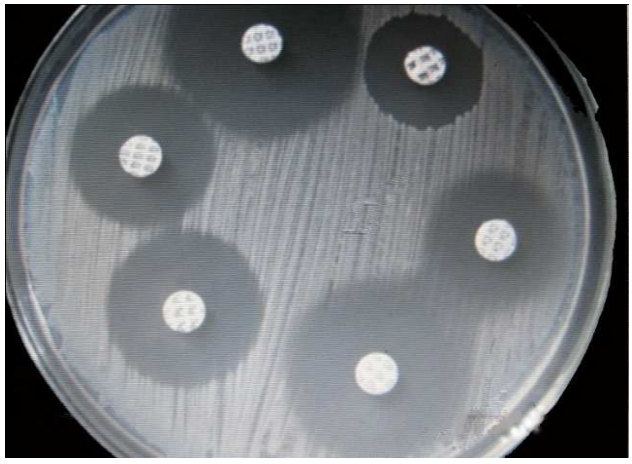
Biochemical Reactions of Klebsiella



Staphylococcus aureus with Methicillin Resistance

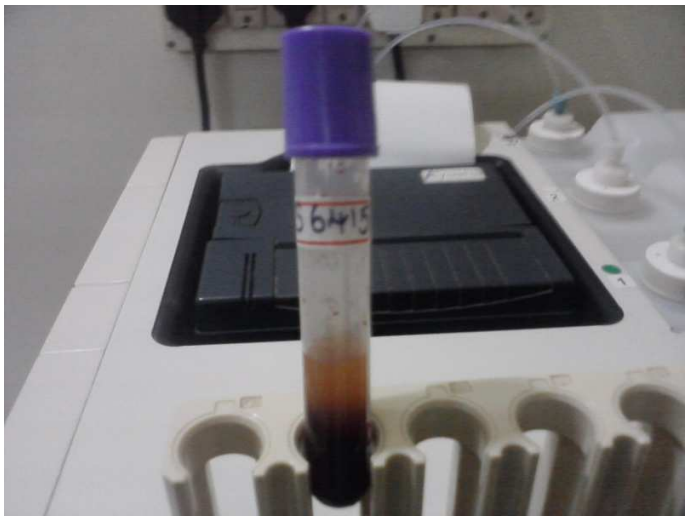


Staphylococcus aureus with Methicillin Sensitivity



$HbA_1C$ Analyser BIO RAD


$HbA_1C$ Analyser with sample



$HbA_1C$ Analyser with Reagents

Diabetic foot ulcers

## DISCUSSION

Worldwide, Diabetic foot lesions are causing major medical, social and economic problems and the leading cause of hospitalization for patients with diabetes[54]. Diabetic foot infection is considered as one of the most threatening and disabling complication for a diabetic patient as the lesions of the extremities can become so severe that the patient may risk the amputation of the toe, foot or leg[6,46].

Because of serious or recurrent infections and impaired healing processes, initially trivial lesion may progress to chronic non healing wounds, gangrene, or untreatable infections that can lead to limb amputation[55]. Many Diabetic foot ulcers are neglected because they may produce few symptoms and their importance is not appreciated by the patients[28]. Patients who develop foot lesions have significantly less knowledge of diabetes including foot care[56].

Hundred pus and wound samples were collected from patients above 20 years of age with known history of Diabetes mellitus, most of the patients belonged to the $5^{th}$ and $6^{th}$ decades of life (37% ) and (28%) respectively .This coincides with the studies listed below by other authors. The mean age of the patients was 59.5 years in Kahn et al study [59], 58 years in Ramani et al study,[28] 58 years in Dipali AC et al study [6]. In contrast the mean age was reported as 75.02 years in NA Pathare et al study [31] and 43 years in study conducted by C.Anandi et.al from Tamil Nadu India[1].

In our study the males were more affected compared to females with a ratio of 2.3:1. This was in concordance with the following studies, D.Vijay et al[21] in 2000 observed a preponderance of male patients showing diabetic foot ulcers (72.5%) compared to female patients (27.5%) The ratio of male to female was 2.6:1. In a study by Dipali AC et al [6] in 2002, 67% of male patients with diabetic foot ulcers were reported against 32.4% of female patients with a ratio of 2.1:1. Prevalence of 58.5% of male patients and 41.2% of female patients with a ratio of 1.41:1 was noted in a study by Fiaz Ur Rehman et al in 2002. Anandi et al[1] 2004 observed a difference of 65.4% and 54.6% among male and female patients with a ratio of 1.2:1. All the above authors have observed a preponderance of males in their study.

In our study most of the ulcers belonged to grade II of Wagner's classification (40) followed by Grade III. The above data correlates with the results published by V.Vijay et al showing 50% grade II ulcers followed by 26.5% grade III Ulcers[21].

Out of the hundred samples 90 yielded aerobic bacterial growth and 10 samples did not yield any growth in our study. In a study by Mohanty et al in 2002, out of the 5,039 pus samples, 2437(48.36%) were culture positive while 1831(33.33%) was culture negative[69].

In the present study the highest average no of isolates per sample was found in Grade 4 ulcers (2.18). In a prospective study of Diabetic foot ulcers conducted by Ekta Bansal Et.al an average of 1.52 isolates per case was reported[11]. But here the maximum number of isolates per case was reported from Grade II. In Uday Kelkar et al study in

2004[33] an average of 3.7 organisms were yielded per sample. The yield from the deep tissue samples was significantly higher than the yield from surface swab samples.

Our study showed 31% of polymicrobial infections similar to Ekta Bansal et al study[11] showing 35% polymicrobial infection. In Contrast polymicrobial growth was noted as 64.4% in a study conducted by C.Aanandi et.al, from Tamil Nadu India[1]. Out of the 427 positive cultures 83.8% were polymicrobial, in a clinical trial conducted by Diane M Ceitron et al, at R.M.Alden Research Laboratories California [55]. It's because the maximum number of patients in these two studies belonged to Wagner's Grade III, but in our study the maximum number of patients with polymicrobial growth were in the Wagner's Grade II.

In our study among the enterobacteriaceae isolates, Proteus mirabilis was the most common isolate (23.2%) followed by E.Coli(16.87%) & Klebsiella8.8% . Citrobacter freundii was the least common isolates belonging to the enterobacteriaceae family which is similar to the study by Uday Kelkar et al(2004)[33] Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella species and Enterococcus species were organisms isolated in decreasing order .

But in Ami Variyae et al study Klebsiella pneumonia (59.7%) was the most common isolate followed by E.coli 40.29% [49] . Similar results were shown in a study conducted by Emily . S. Bomasang et al. with 45.8% of E. coli[45].

In a study by Ashwin N Anantha Krishnan Et al. 21 % of E. coli were isolated [38] . This difference in common isolate in different studies might be due to different grade of ulcers selected. In our study among the non enterobacteriaceae Pseudomonas (16%) was

the highest isolate, Acinetobacter species was the least common isolate (2.4%). In a study conducted by Vishwanath et al[51] Pseudomonas species was accounting for 17% of the isolates which is similar to our study. Pseudomonas aeruginosa was the most common isolate accounting for 21.7% in Ekta banzal et al study[11].

Staphylococcus aureus showing sensitivity of 26% to Cefotaxime, 47.8% to Ciprofloxacin and 52% to Ofloxacin and Amoxyclav. CONS isolates are 66% sensitive to Amikacin and 100% sensitive to Vancomycin. And all these isolates show 100% sensitivity to Vancomycin and Linezolid. Out of the 23 Staphylococcus aureus isolates 12 isolates were methicillin resistant (55%) and 11 isolates were found to be MSSA (45%).In a study by C.N. Dang et al [42] MRSA was 30.2 %. 42.86 % of MRSA was seen in a study conducted by Murugan S. et al [66] while assessing the prevalence of MRSA among diabetic Ulcer patients which correlates with our study .

In our study MRSA were 100 % resistant to Ampicillin, 65% to Erythromycin & 70% to Cephalexin .100% sensitivity was noted to Vancomycin and Linezolid. In a study by Sivaram Uma Devi Et al. 65% of the 29 Staphylococcus aureus isolates were found to be methicillin resistant [66]. Resistance to Penicillin was 100%, Erythromycin was 31 %. and Gentamicin was 59%. Sensitivity was higher to Vancomycin. They were of the opinion that combination of Vancomycin and Linezolid for coverage of Gram Positive Cocci could be used empirically and then tailored to the needs of the individual once susceptibility testing report was available.

In our study, Proteus sp (23.2%) was the major gram negative aerobe isolated which showed 100% sensitivity to Piperacillin ,84% to Cefaperazone with sulbactum and Ceftrioxone, 76% sensitivity to Cefotaxime,53% to Ciprofloxacin and for amikacin 46 % sensitivity. It showed lowest sensitivity to Ampicillin(15%). In a study by Ekta bansal et al[11] in 2009 Proteus sp exhibited 100% sensitivity to Cefaperazone with sulbactum and Ceftrioxone, and amikacin. It showed lowest sensitivity to Amoxicillin( 33%). In a study by Vimalin Hena et al[52] in 2010 the proteus isolate was 71% sensitive to Ciprofloxacin 57% to Amikacin.

In our study Pseudomonas sp showed 100% sensitivity to Meropenem followed by 94% to Piperacillin with tazobactum ,68% to Cefaperazone with sulbactum , 52% to Ceftazidime ,47% to Ciprofloxacin, Amikacin and Gentamicin showed 10.5% sensitivity. In a study by Ekta bansal et al in 2009 Pseudomonas showed 100% sensitivity to Imipenem , 94% to to Ceftazidime, 83% to Piperacillin 63% to Ciprofloxacin. For Amikacin 79% and Gentamicin 33% sensitivity was noticed[11] . In a study by Vimalin Hena et al in 2010 study Pseudomonas sp showed 100% sensitivity to Imipenem followed by 83% to Piperacillin, 41% to Ceftazidime and 22% to Ciprofloxacin [52].

In our study Escherichia coli showed highest sensitivity to Piperacillin with tazobactum and Cefaperazone with sulbactum , 82% to amikacin , 76% to Cefotaxime and Ceftriaxone (70%) and 47% to Ceftazidime. In a study by Ekta bansal et al in 2009 study Escherichia coli showed 96% sensitivity to Cefaperazone with Sulbactum ,

90% to Amikacin , 82% to Ceftazidime and 33% to Ciprofloxacin[11] . In a study by Vimalin Hena et al in 2010 study Escherichia coli showed 71% sensitivity to Piperacillin followed by 65% to Ceftazidime. For Amikacin, Gentamicin and Cefotaxime 59% sensitivity was noticed [52].

Anandi et al [1] observed that all the aerobes were sensitive to amikacin and gentamicin except two Pseudomonas sp isolates. All the aerobes were susceptible to Cefotaxime except four Pseudomonas sp isolates which were susceptible to amikacin and gentamicin. Dipali AC et al [6] found that more than 70% of the aerobic gram negative bacilli were sensitive to aminoglycosides, amikacin (95.74%) and gentamicin (70.21%). Sensitivity to Cefotaxime was 63.50%. Nema et al [67] found that the gram negative bacilli were most sensitive to aminoglycosides and sensitivity to Cefotaxime was 63.12%.

In our study only aerobic growth of organism were analyzed. Higher grade of diabetic foot ulcers have known to be associated with mixed flora comprising of both aerobes and anaerobes. When antimicrobial therapy is indicated for treatment of diabetic foot ulcers the likelihood of complex aerobic and anaerobic flora should be considered and appropriate antimicrobial agents selected.

As per our study maximum no of patients (45) with DFI had HbA1C levels more than 10.The number of patients with having HbA1C above 8 are 76 %. In a study by Nahid Rouhipour[59] 62.9% of patient had poor diabetes control (HbA1C of 8% or higher) and in a study by M.B. Girish et al the mean glycated hemoglobin was 7.80 ± 0.80[74]. The patients who underwent amputation presented a significantly higher incidence of ischemic diabetic foot with, HbA1C > 7. As per Wheat et al study the majority of

patients with the diabetic foot ulcers had bad control diabetic status ($HbA_1C > 8.5$) [16]. Among 183 diabetic individuals treated at the Johns Hopkins Wound Center Mean HbA1c was 8.0 [72]. In Strhova L et al study [62] in 2006 significant number (65%) of infected ulceration on feet was reported in poorly controlled diabetic patients with $HbA_1C$ above 8. Infection and osteomyelitis together remains as significant risk factor for amputation. In this study $HbA_1C$ appears to be significant predictor for amputation.

But in Nighat Akbar et al's study [73] though the mean value of glycosylated haemoglobin (Hb) was 8.2 (6 - 16.6), 75% of patients showed an HbA1c level <8.0; in 25% cases, it was >8. All the patients who had an $HbA_1C$ level >10% manifested with various types of foot lesions.

In our study maximum number of cases (45) were recorded with $HbA_1\mathbf{C}$ levels of >10 and most number of cases were found in Grade II and Grade III. The total distribution of mono microbial and polymicrobial growth was 62 and 28 respectively with the maximum number of growth recorded in $HbA_1\mathbf{C}$ levels of more than 10. In Shaba Tiwari et al study[71] $HbA_1\mathbf{C}$ was same in polymicrobial and the mono-microbial infections (9.9% versus 9.5%; p = 0.1), of diabetic foot patients.

There was no relationship between the bad control diabetic status and the type of pathogen isolated from the ulcers similar as per Wheat et al study[16].

In our study the MRSA and MSSA Isolates are evenly found in all the Categories of Hb $A_1C$ levels. MRSA constitutes 50% in $HbA_1\mathbf{C}$ Levels of <8 and 8 to 10 and 55% in $HbA_1\mathbf{C}$ Levels of > 10.

As this is a pilot study regarding the correlation of $HbA_1$**C** with Wagner's grade of DFI and MRSA more research and detailed knowledge is needed in future to assess the appropriate management of DFI patients. In our study the incidence of diabetic foot lesions strongly correlates with the poor glycemic control, which in itself is best manifested by the levels of glycosylated Hb.

## Summary

The present study was carried out in the Department of Microbiology, CMC from March 2009 to Sep 2010 to look for the pattern of growth of aerobic organisms in diabetic foot ulcers.

- Of the 100 DFI cases studied, most of the patients belonged to the $5^{th}$ and $6^{th}$ decades of life (37%) and (28%) respectively.
- Males were more affected compared to females with a ratio of 2.3:1.
- Maximum number of patients were seen in Wagner's Grade II (40 nos), followed by Wagner's Grade III (38nos).
- Maximum average aerobes per sample were found in Grade 4 ulcers (2.18).
- Out of the hundred samples assessed for the growth of aerobic organisms. 90 yielded aerobic bacterial growth 10 samples did not yield any growth.
- The average number of microorganism /sample is decreasing as the Wagner's grade decreases.
- The number of isolates are more than the number of samples and the average number of microorganism /sample is more than one because of polymicrobial growth yield respectively.
- Out of the 90 culture positive samples mono microbial growth was found in 62 and 28 samples yielded polymicrobial growth with percentage of 69 and 31.

- Among Gram positive aerobes, Staphylococcus aureus was the predominant isolate (18.4**%**). Among Gram negative aerobes, Proteus sp was the most common isolate (23.2%) followed by E.Coli 16.8% and Pseudomonas 16%. Acinetobacter species was the least common isolate (2.4%).

- The Staphylococcal isolates show 100% sensitivity to Vancomycin and Linezolid. Staphylococcus aureus showed 50% Methicillin resistance(MRSA). Coagulase negative Staphylococci showed 66% sensitivity to Amikacin.

- Proteus sp showed 100% sensitivity to Piperacillin, 84% to Cefaperazone with sulbactum and Ceftriaxone, 76%. Sensitivity to Cefotaxime, 53% to Ciprofloxacin and 46% to Amikacin.

- Pseudomonas sp showed 100% sensitivity to Meropenum followed by 94% to Piperacillin with tazobactum, 68% to Cefaperazone with sulbactum, 52% to Ceftazidime, and 47% to Ciprofloxacin. Amikacin and Gentamicin showed 10.5% sensitivity.

- Escherichia coli showed highest sensitivity to Piperacillin with tazobactum and Cefaperazone with sulbactum, 82% to amikacin , 76% to Cefotaxime, Ceftriaxone (70%) and 47% to Ceftazidime.

- Maximum number of Diabetic foot ulcer cases (45) were recorded with $HbA_1$**C** levels of >10 .

- The total distribution of Mono microbial and polymicrobial growth was 62 and 28 respectively with the maximum number of growth recorded in $HbA_1$**C** levels of more than 10

- MRSA constitutes 50% in $HbA_1$**C** Levels of <8 and 8 to 10 and 55% in $HbA_1$**C** Levels of > 10.

## CONCLUSION

Diabetic foot ulcers are one of the most common and dreaded complications of Diabetes mellitus. It is more common among males in the 5th and 6th decades of life. As the Wagner's grade increased the prevalence of infections also increased. Mono microbial infections prevailed over polymicrobial infections.

While staphylococcus aureus was the most common among gram positive cocci, Proteus species was the most common isolate among the gram negative pathogens.

The Gram negative bacterial isolates were highly sensitive to Piperacillin with tazobactum followed by Cefaperazone with sulbactum. Pseudomonas aeruginosa showed highest sensitivity to Meropenem. Staphylococcus aureus showed 55% Methicillin resistance ( MRSA).

Foot problems in diabetes continue to persist and will be challenging the clinicians. They can be properly treated by proper and prompt antibiotic therapy to optimize patient care and to improve clinical outcome.

The incidence of diabetic foot lesions strongly correlates with poor glycemic control which in itself is best manifested by the levels of glycosylated hemoglobin levels.

There was significant association between DFI and higher $HbA_1C$ Levels. But no correlation found between $HbA_1C$ levels and the polymicrobial nature of infection and prevalence of MRSA in DFI.

Further research is needed to study about correlation of $HbA_1C$ levels with other factors in DFI and important studies need to be performed to overcome the serious problem of foot ulceration in diabetes mellitus.

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## ANNEXURE I

**PROFORMA**

Name: Date:

Age: Case no. :

Sex: OP no. :

**CLINICAL HISTORY AND EXAMINATION**

Duration of diabetes:

Type of diabetes:

Smoking:

Hypertension:

Duration of foot ulcer:

Antibiotic treatment:

Foul smell:

Fever:

Crepitations:

Purulent discharge:

Vasculopathy:

Neuropathy:

Osteomyelitis:

Cellulitis:

Gangrene:

**INVESTIGATIONS**

Blood sugar levels:

Foot X-ray: if needed

HbA1c level -

**Culture of wound swab samples aerobically**

• Blood agar

• MacConkey agar

• Nutrient agar

**Biochemical reactions:**

**Organisms isolated:**

**Antibiotic susceptibility pattern of the isolates**

**GRAM POSITIVE AEROBES**

Ak, Lz, Of, Ac, Cot, Cfs, Cp, E, G, Ci, Cip, Ce, Do, Cn

**GRAM NEGATIVE AEROBES**

Pit, Ac, Mrp, Cfs, Ak, Caz, Gm, Of, Ci, Ctx, Cot

Organism isolated:

**REPORT**:

# Appendix II

**OXIDASE TEST**

A strip of filter paper was soaked with a little freshly made 1% solution of tetramethyl para phenylene diamine dihydrochloride. A speck of the culture was rubbed on it with a wooden stick. An intense deep purple hue developing within 10 seconds was taken as positive reaction.

Positive control - Pseudomonas aeruginosa

Negative control - Escherichia coli

**INDOLE TEST**

Medium- peptone water

**Procedure**

Pure single colony was inoculated into peptone water and incubated at $37^{o}$C for 18-24 hours. 0.2 ml of kovacs reagent (paradimethyl aminobenzaldehyde) was added to culture broth.

**Interpretation**

A positive reaction was indicated by the formation of pink ring at the junction.

**METHYL RED TEST**

**Medium**- MR broth

**Reagent** – Methyl red

**Procedure**

MR broth was inoculated with a pure culture of the test organism and incubated for 48 hrs at $37^0$ C. Then 5 drops of methyl red reagent was added to the broth.

**Interpretation-**A stable red color on the surface of the medium indicates a positive test

Positive control – Escherichia coli

Negative control – Enterobacter aerogenes.

**VOGES – PROSKAUER TEST**

**Medium -**VP broth

**Reagents** – VP reagent A – 5% alpha naphthol

VP reagent B -40% Potassium hydroxide

**Procedure**

A tube of VP broth was inoculated with a pure culture of the test organism and incubated for 24 hours at $37^0$ C. At the end of this time, 1ml of the aliquot is taken into a clean test tube and 0.6 ml of 5% α- Naphthol is added followed by 0.2 ml of 40 % KOH in that order and the tube was shaken gently. Then the tube is allowed to remain undisturbed for 10-15 minutes.

**Interpretation**-Red color indicates a positive reaction.

Positive control – Enterobacter aero genes

Negative control – Escherichia coli.

**CITRATE UTILISATION**

**Medium-** Simmons citrate medium

**Procedure** - The entire surface of the slant was inoculated lightly from a young

culture and incubated at $37^0$ C for 24-48 hours.

**Interpretation**

The test was considered positive when the medium turned deep blue in color along with growth on the surface.

Positive control - Klebsiella pneumoniae

Negative control- Escherichia coli

**UREASE TEST**

**Medium** – Christensen's Urease medium (Refer Annexure II)

**Procedure**

The medium was inoculated with the test organism and incubated at $37^0$C overnight.

**Interpretation**

Development of pink colour throughout the medium was taken as positive test and yellow colour as negative test.

Positive control – Proteus vulgaris.

Negative control – Escherichia coli

**TRIPLE SUGAR IRON AGAR**

**Medium -** TSI agar

**Procedure**

The center of the butt of TSI agar is stabbed with a straight wire charged with test organism and the slant is streaked and incubated overnight.

**Interpretation**

Acid butt (yellow) / Alkaline slant (red) -Glucose fermented

Acid butt (yellow)/ Acid slant (yellow) - Glucose and lactose/sucrose fermented

Gas bubbles in butt- gas production

Blackening in butt- Hydrogen Sulphide produced

Alkaline slant / Alkaline butt- No sugars fermented

**CARBOHYDRATE UTILIZATION TEST**

**Medium**

**S**ugar medium containing 1 gram sugar in 100 ml nutrient broth base with bromothymol blue as indicator.

**Procedure**

Test organism was inoculated into each sugar medium and incubated subsequently for 18 to 24 hours.

**Interpretation**

Positive test was shown by yellow coloration of the medium due to acid production. Yellow colouration indicates acid production due to fermentation.

## Abstract

### Background:

Diabetes mellitus is a progressive disease, diabetic foot is the major complication of it, and eventually leads to development of gangrene and lower extremity amputation. This study has been carried out to detect the antibiotic sensitivity pattern of the isolates in relation to $HbA_1C$ levels.

### Objectives:

To study the prevalence of diabetic foot ulcers in various age groups and gender.
To isolate and identify the bacterial isolates causing diabetic foot infections.
To analyze $HbA_1C$ levels in relation with Diabetic Foot Infections, bacteriological profile and antibiotic susceptibility pattern.

### Materials and method:

Pus and wound swabs were collected from around 100 diabetic patients with foot ulcer attending the Surgery Out-Patient Department of Coimbatore Medical College Hospital. The samples received in the Department of Microbiology were processed for aerobic culture and antibiotic sensitivity testing during the study period. Blood samples were collected to analyze the $HbA_1C$ levels.

### Results

Of The 100 cases studied, most of the patients belonged to the $5^{th}$ and $6^{th}$ decades of life (37%) and (28%) respectively. Males were more affected compared to females with a ratio of 2.3:1.

Maximum number of patients with Diabetic Foot Ulcers were seen in **Wagner's** Grade II (40 nos), followed by 38 DFI patients in Wagner's Grade III. Among Gram positive aerobes, Staphylococcus aureus was the predominant Isolate (18.4%). Among Gram negative aerobes, Proteus spp was the most common isolate (23.2%) followed by E.Coli 16.8% and Pseudomonas 16%. Acinetobacter species was the least common isolate (2.4%).

While staphylococcus aureus was the most common among gram positive cocci, Proteus species was the most common isolate among the gram negative pathogens.

### Conclusion

Staphylococci and Proteus were the two most common isolates detected in diabetic foot infections. There was significant association between DFI and higher $HbA_1C$ Levels. But no correlation found between $HbA_1C$ levels and the polymicrobial nature of infection in DFI.